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INHERITANCE IN A "CONSTANT" HYBRID BETWEEN AEGILOPS OVATA AND TRITICUM DICOCCUM[1]

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INTRODUCTION

In intergeneric hybrids between certain species of Aegilops and Triticum the F_1 form tends to reappear exclusively in the later generations. This so-called "constant" hybrid form combines the characters of both parents, but is so different from either of them that it has even been suggested that it deserves to rank as a new species. Furthermore, the behavior in these crosses has been cited as evidence supporting the view that wheats with 21 chromosomes (haploid) originated as the result of crossing between a species of Aegilops and a species of Triticum with 14 chromosomes.

From publications on Aegilops-Triticum hybrids it appears that their cytology has received more study than their morphology. It is the purpose of this paper to report the phenotypic behavior of the descendants of the cross between *A. ovata* and *T. dicoccum*, with special reference to the constancy of the F_1 form. The data presented show that although there is a high degree of constancy in the later generations, measurable and even marked departures from the F_1 type may occur.

REVIEW OF LITERATURE

Tschermak and Bleier (*8*)[3] report a constant-hybrid form of inheritance resulting from crosses of *Aegilops ovata* with *Triticum dicoccoides* and with *T. durum*. Forms varying in fertility from entirely sterile to fairly fertile appeared in the later generations. Off-type forms, usually sterile, occasionally appearing in F_3 and later generations of the cross *A. ovata* × *T. durum* were attributed to natural crossing. All generations of the cross *A. ovata* × *T. dicoccoides* were entirely of the F_1 form. The phenomenon of nonsegregation was explained by cytological studies of the F_5 and F_6 individuals, which showed 28 haploid chromosomes, that is, double the number of haploid chromosomes present in any of the above parents, and usually 56 chromosomes in the somatic cells. Regular reduction division in the pollen mother cells was found, but it was further observed that frequently in the cytoplasm there were strongly stained bodies which might be extruded chromosomes. When, however, F_5 or F_6 individuals of the cross *A. ovata* × *T. dicoccoides* were crossed with F_5 or F_6 individuals of the cross *A. ovata* × *T. durum* the haploid chromosome number of the new hybrid was 28 and the reduction divisions were quite normal. In later investigations by

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[3] Reference is made by number (italic) to Literature Cited, p. 679.

Bleier (*1*), *A. ovata* was crossed with F_5 or F_6 plants of the crosses *A. ovata* × *T. dicoccoides* and *A. ovata* × *T. durum*. The F_1 individuals of these crosses contained 14 bivalent and 14 univalent chromosomes.

Sax (*7*) found 28 haploid chromosomes in the F_1 of the cross *Aegilops ovata* × *Triticum dicoccum*, no pairing of chromosomes occurring. Percival (*6*) found little if any pairing of chromosomes in his study of the cross *A. ovata* × *T. dicoccum*.

Longley and Sando (*5*) examined cytologically four F_3 plants of the cross *Aegilops ovata* × *Triticum dicoccum*. Three individuals had 25, 26, and 27 haploid chromosomes, respectively. No definite count was established in the fourth individual, but 5 or 6 lagging chromosomes were observed. Univalent chromosomes were commonly seen.

Kagawa (*2*) found 28 chromosomes in the root tips of an F_1 plant of *Triticum durum* × *Aegilops ovata*. Dyad cells and giant microspores were observed in the F_1 meiosis.

MATERIAL AND METHODS

Aegilops ovata, a native grass of southern Europe and western Asia, is a low-growing annual which under native conditions is about 25 to 50 cm. tall, with slender bent culms. Under greenhouse culture *A. ovata* averaged 74 cm. in height. The leaves of the variety used in these experiments are narrow and hairy. The head consists of from 3 to 5 spikelets, each of these usually with 3 or more flowers. The glumes are white, rounded, many nerved, and roughly scabrous, with from 4 to 7 awns, the longest of these being 3.2 cm. The lemma is lightly scabrous, with from 1 to 4 awns or teeth, the longest awn averaging 2.2 cm. At maturity the head is deciduous as a unit.

Black Winter emmer, one of the cultivated varieties of *Triticum dicoccum*, was introduced into this country from France in 1904. It is erect in growth and averaged 118 cm. in height in the greenhouse. The culms are thick and the leaves wide and puberulent. The head is very dense and contains an average of 26 spikelets, each usually with two flowers. The glumes are distinctly keeled, narrow, black, and pubescent, each glume having a beak less than 2 mm. in length. The lemma is black and pubescent, with one awn averaging 12.1 cm. in length. Each spikelet of the emmer is deciduous at maturity at the base of the pedicel, which usually remains attached to the base of the spikelet which it bears.

The parent and hybrid plants were grown both in the greenhouse and in the field at the Arlington Experiment Farm, near Washington, D. C. In Figure 1 are shown representative plants of the two parents and the F_1 hybrid.

The F_1, F_2, and F_3 plants used as parents of the following generation were selfed by inclosing the head in a glassine bag or by the isolation of single plants from all relatives. As attempts to cross different plants of the same generation rarely gave seed, isolation or bagging to insure selfing was discontinued in the F_4. In the F_6 population four natural crosses with *Triticum vulgare* were evident, these being the only cases of natural crossing observed.

The usual technic employed in crossing wheat was used. Well-developed flowers were emasculated and the pollinations were made when the flowers were judged to be receptive.

FIGURE 1.—A, *Aegilops ovata;* B, F_1 *A. ovata* × *Triticum dicoccum;* C, *T. dicoccum* (Black Winter emmer). (One-twelfth natural size)

The principal aim of the study was to measure the degree of segregation occurring in the F_2 and later generations. Certain characters introduced by the parents and distinguishable by the eye or touch, in which segregation could be expected, were selected for study. In addition, data on nine other characters of a quantitative nature were recorded for each plant. The influence of the seasonal conditions was partly overcome by repeating the cross with the same strains of *Aegilops ovata* and *Triticum dicoccum* and growing two generations in the greenhouse under comparable conditions in the same year.

A description of the quantitative characters follows.

DESCRIPTION OF CHARACTERS

Days to heading.—The number of days from seeding until the first head was visible. This character varied greatly with the time of seeding, and the comparisons are made between plants and generations sown the same day and year.

Height of plant.—The height of the tallest culm, in centimeters.

Length of head.—The length of the primary head from the base of the lowest perfect spikelet to the top of the apical spikelet, exclusive of awns, in centimeters.

Number of spikelets.—The number of developed spikelets on the primary head.

Largest number of awns on glume.—The largest number of awns more than 2 mm. long on any glume of the primary head.

Smallest number of awns on glume.—The smallest number of awns more than 2 mm. long on any glume of the primary head.

Length of longest awn on glume.—The length of the longest awn on the glume of any spikelet of the primary head, in centimeters.

Length of longest awn on lemma.—The length of the longest awn on the lemma of any spikelet in the primary head, in centimeters.

Fertility percentage.—The percentage of the developed flowers of the primary head of a plant which set seed. No rudimentary flowers were considered in obtaining the fertility data.

EXPERIMENTAL DATA

The first hybrids between *Aegilops ovata* and Black Winter emmer, hereafter referred to as Aegilops and emmer, respectively, were made in the greenhouse in 1922. Twenty-four flowers of the Aegilops were pollinated with the emmer. Six seeds resulted and six F_1 plants were matured in 1923. Four of the F_1 plants set one or more seeds and an F_2 population of seven plants was grown in the greenhouse in 1924. These seven plants were of one general phenotype similar to the F_1. The progeny of the F_2 plants that produced seed formed the populations used for the study of the F_3 and later generations.

A larger F_2 population appeared essential, and it was desirable to study also two generations of the cross grown the same year under comparable environmental conditions. Therefore, the cross was repeated, reciprocally, in 1925. A new F_1, consisting of 67 plants, resulted from these pollinations. These plants and an F_4 population of 44 individuals from the crosses of 1922 were grown in the greenhouse in 1926 under comparable conditions. The F_4 population was grown from seed of 26 F_3 plants differing phenotypically from one another. The F_5 was grown from 15 F_4 plants chosen as representing the range of variation in the F_4 generation. The F_2 from the crosses of 1925 was grown in the greenhouse along with the F_5 in 1927. Beginning with the F_4 generation, the effect of selection for phenotypic differences should be shown in the data on the hybrid population, provided the differences are also genotypic in character.

COMPATIBILITY OF THE PARENTAL TYPES TO CROSSING

The hybridizing of Aegilops and emmer is readily accomplished when Aegilops serves as the female parent. The cross between Aegilops and emmer gives a much higher percentage of hybrid seed when Aegilops serves as the female parent than when the reciprocal combination is made, as may be seen from the data presented in Table 1.

TABLE 1.—*Flowers pollinated and seeds obtained in reciprocal F_1 hybrids between Aegilops ovata and Triticum dicoccum (Black Winter emmer)*

Hybrid *	Year	Place	Flowers pollinated	Hybrid seeds obtained	
			Number	*Number*	*Per cent*
A. ovata × T. dicoccum	1922	Greenhouse	24	6	25
Do	1925	Field	209	109	52.2
Do	1926	Greenhouse	24	12	50
Total			257	127	49.4
T. dicoccum × A. ovata	1925	Field	255	22	8.6
Do	1926	Greenhouse	24	3	12.5
Total			279	25	9

* The female parent is placed first in combinations here and elsewhere.

An average of 49.4 per cent of hybrid seed was obtained when the Aegilops was fertilized by the emmer, as compared with an average of 9 per cent in the reciprocal combination.

To reduce the effect of differential receptivity of the greater number of flowers of the emmer head as compared with the fewer number of flowers of the Aegilops head, 50 flowers of both emmer and Aegilops were pollinated and repollinated with Aegilops and emmer pollen, respectively, on three successive days. The number of hybrid seeds obtained on the emmer heads amounted to approximately 10 per cent as compared with 62 per cent on the Aegilops heads. This difference in response in reciprocal crosses is commonly encountered in hybridizing Aegilops species and wheat species, as shown by Leighty, Sando, and Taylor (*4*).

RECIPROCAL F_1 HYBRIDS

Although different amounts of hybrid seed were obtained in the reciprocal crossing of Aegilops and emmer, the resulting reciprocal F_1 hybrid plants differed only slightly in respect to the eight characters studied. No differences that can be considered significant are shown by the measurements given in Table 2.

TABLE 2.—*Average data on plant and head characters of reciprocal F_1 hybrids between Aegilops ovata and Triticum dicoccum*

Hybrid	Height of plant	Days from planting to heading	Length of head	Spikelets per head	Largest number of awns on glume	Smallest number of awns on glume	Length of longest awn on glume	Length of longest awn on lemma
	Cm.	*Number*	*Cm.*	*Number*			*Cm.*	*Cm.*
A. ovata × T. dicoccum	102.7±0.53	107.2±0.39	8.03±0.13	8.33±0.32	3.0±0	2.0±0	5.73±0.10	6.73±0.13
T. dicoccum × A. ovata	103.7±1.41	106.2± .42	8.24± .18	8.00± .18	3.0±0	2.0±0	6.17± .08	7.14± .13

FIGURE 2.—A (left to right), heads from F_1, F_3, F_2, and F_4 plants; B, *Aegilops ovata* and *Triticum dicoccum*. (About two-thirds natural size)

F_1 HYBRID AND PARENTS

Data on certain prominent plant characters of the Aegilops and emmer parents and the F_1 hybrid are given in Table 3 and heads are shown in Figure 2.

TABLE 3.—*Descriptive data*[a] *on Aegilops ovata, Triticum dioecoum, and F_1 hybrid*

Character	A. ovata	F_1 hybrid	T. dicoccum
	Mean PE	*Mean PE*	*Mean PE*
Height of plant (centimeters)	74.0±0.70	104.3±0.61	118.0±1.40
Days from planting to heading (number)	101.3±1.27	111.0± .33	116.8±1.85
Tillers per plant (number)	14.7± .45	5.4± .17	1.8± .42
Length of head (centimeters)	3.2± .03	8.0± .06	8.5± .18
Spikelets per head (number)	3.1± .07	8.0± .07	26.0± .38
Largest number of awns on glume	6.0± .00	3.0± .00	Beak only.
Smallest number of awns on glume	3.9± .07	2.0± .01	Beak only.
Length of longest awn on glume (centimeters)	3.2± .03	6.4± .03	Beak only.
Length of longest awn on lemma (centimeters)	2.2± .04	7.6± .05	12.1± .20
Glumes scabrous or nonscabrous	Scabrous.	Scabrous.	Nonscabrous.
Glumes pubescent or nonpubescent	Nonpubescent.	Pubescent.	Pubescent.
Glumes black or white	White.	Black.	Black.
Sheath and leaves hairy or puberulent	Hairy.	Hairy.	Puberulent.
Outer glume rounded or keeled	Rounded.	Keeled.	Keeled.
Awns spreading or upright	Spreading.	Intermediate.	Upright.
Dehiscence of spike	As a unit.	As a unit.	As spikelets.
Manner of growth	Prostrate.	Intermediate.	Erect.

[a] Obtained on 67 F_1 plants and on 10 plants of each parent, grown in the greenhouse in 1926.

The F_1 hybrid approaches the emmer parent in the more prominent vegetative characters, such as growth habit, number of tillers, length of head, and the presence of black, pubescent chaff. The detailed head characters indicate that the F_1 awn, spikelet, and glume characters are either intermediate or are more like the Aegilops. The glume of the Aegilops may have as many as six awns, while the emmer glume has only a beak 2 mm. or less in length. The F_1 had two or three awns on each glume, and the longest of these was almost twice as long as the longest awn of the Aegilops. The longest awn of the lemma of the F_1 was intermediate as compared to the parental types.

F_1 AND LATER GENERATIONS

The F_2 population of 1924 consisted of seven individuals. All seven were of the F_1 type except that one plant had more spreading awns than the others. The F_3 generation grown in 1925 consisted of 91 plants, descendants of the seven F_2 individuals of 1924. In the F_3 generation phenotypic differences occurred, such as lax and compact spikes, spreading and upright awns, long and short awns, and tall and short stature. Plants representing these extremes were among those selected as parents for the F_4 generation.

The means and coefficients of variation for six or eight quantitative plant characters of the mass F_1, F_2, F_3, F_4, and F_5 generations are given in Table 4. In addition, the data for these same characters are given for four selected families, two in the F_4 and two in the F_5.

These characters are influenced more or less by environment, and a comparison of one generation with another is preferable when both have been grown the same year under conditions as nearly comparable as possible. Such conditions exist for a comparison of the F_1 with the F_4 and of the F_2 with the F_5, as these two pairs of generations, respectively, were grown in the greenhouse in the same year.

TABLE 4.—*Biometrical constants on eight plant characters of five generations of the cross Aegilops ovata × Triticum dicoccum*

Generation	Year grown	Individuals	Days from planting to heading		Height of plant		Length of head		Spikelets per head	
			Mean	CV	Mean	CV	Mean	CV	Mean	CV
		Number	*Number*		*Cm.*		*Cm.*		*Number*	
F_1	1926	67	111.0±0.33	3.6±0.21	104.3±0.61	7.1±0.41	8.04±0.06	8.8±0.51	7.97±0.07	9.9±0.58
F_2	1927	71	129.4± .47	4.5± .25	91.6±1.04	14.2± .83	6.45± .09	18.3±1.08	6.76± .08	14.6± .6
F_3	1925	91					7.03± .08	15.2±1.03	6.75± .06	13.0±1.02
F_4	1926	44	120.8± .78	6.3± .48	91.3± .79	8.5± .62	7.68± .11	14.7±1.09	8.09± .09	11.1± .81
F_5	1927	99	132.4± .27	3.0± .14	94.5± .83	13.0± .63	7.09± .09	18.6± .93	7.22± .07	14.8± .72
F_4 69 [a]	1926	29					6.77± .10	11.8±1.06	8.03± .11	11.1±1.00
F_4 66 [a]	1926	56					6.07± .07	13.0± .84	7.14± .09	13.9± .90
F_5 19 [b]	1927	13	132.0± .07	.3± .04	104.3±1.87	9.6±1.27	8.43± .10	6.0± .79	7.23± .08	5.8± .77
F_5 10–3 [b]	1927	16	131.1± .46	2.1± .25	89.9±1.16	7.6± .91	5.78± .14	14.4±1.75	6.44± .12	11.0±1.33

Generation	Year grown	Individuals	Largest number of awns on glume		Smallest number of awns on glume		Length of longest awn on glume		Length of longest awn on lemma	
			Mean	CV	Mean	CV	Mean	CV	Mean	CV
		Number					*Cm.*		*Cm.*	
F_1	1926	67	3.00±0		1.97±0.01	8.6±0.50	6.35±0.03	6.3±0.37	7.56±0.05	8.6±0.50
F_2	1927	71	3.89± .05	16.5±0.96	2.28± .04	21.1±1.24	4.56± .07	18.2±1.08	5.71± .08	17.0± .99
F_3	1925	91	3.62± .04	15.6±1.02	2.05± .02	15.1±1.02	4.96± .05	15.3±1.02	5.69± .07	16.3±1.03
F_4	1926	44	3.98± .08	19.4±1.48	2.25± .04	19.2±1.43	5.16± .08	15.3±1.13	6.61± .09	13.5± .99
F_5	1927	99	3.99± .05	19.5± .97	2.53± .04	25.3± .87	5.31± .08	23.2± .67	6.12± .08	19.0± .95
F_4 69 [a]	1926	29	3.07± .03	8.5± .75	1.90± .04	15.8±1.40	3.73± .07	15.0±1.36	5.81± .09	11.9±1.05
F_4 66 [a]	1926	56	3.00± .04	14.0± .91	1.84± .03	19.6±1.30	4.63± .05	12.1± .78	6.17± .07	12.2± .78
F_5 19 [b]	1927	13	3.92± .14	18.6±2.54	2.85± .07	12.3±1.65	7.05± .14	10.8±1.45	7.37± .17	12.6±1.72
F_5 10–3 [b]	1927	16	3.94± .09	14.2±1.73	2.00±0		5.99± .12	11.5± .98	5.86± .10	10.4± .64

[a] This line is from an F_3 plant and was grown in the field. Data on this are not included in F_4 data above.
[b] This line is from an F_4 plant but was grown in the greenhouse. Data on this are included in F_5 data above.

Table 5 shows the differences between the means of each of the eight plant characters in the F_1 and the F_4 and in the F_2 and the F_5 grown, respectively, under comparable conditions.

TABLE 5.—*Differences between the means of eight plant characters in the F_1 and F_4 and in the F_2 and F_5 of the cross Aegilops ovata × Triticum dicoccum*

Characters	Difference between means of generations—	
	F_1 and F_4 [a]	F_2 and F_5 [a]
Days from planting to heading (number)	9.8 ±0.85	3.0 ±0.54
Height of plant (centimeters)	13.0 ±1.00	2.9 ±1.33
Length of head (centimeters)	.36± .13	.55± .13
Spikelets (number)	.12± .11	.46± .11
Largest number of awns on glume	.98± .08	.10± .07
Smallest number of awns on glume	.28± .04	.25± .06
Length of longest awn on glume (centimeters)	1.19± .09	.75± .11
Length of longest awn on lemma (centimeters)	.95± .10	.41± .11

[a] Grown under comparable environmental conditions.

The means of the F_4 are significantly different from those of the F_1 in at least six of the eight characters. Differences in length of head and number of spikelets can not be considered significant. The F_1 plants averaged 13 cm. taller and almost 10 days earlier than the average F_4 individual. The largest number of awns on the glume averaged 3.98 in the F_4 and 3.0 in the F_1. The longest awn on the glume averaged 1.19 ± 0.09 cm. shorter in the F_4 than in the F_1. The longest awn on the lemma averaged 0.95 ± 0.10 cm. shorter in the F_4 than in the F_1. The shorter plant, larger number of awns on the glume, and shorter awns on both the glume and the lemma of the average F_4 plant, as compared with the average F_1, show greater resemblance to the Aegilops parent than is found in the F_1. The date of heading of the F_4, however, approached more closely that of the emmer parent than did the F_1.

The F_2 and F_5 generations were grown in the greenhouse in 1927. All kernels borne on the F_1 plants of 1926 that were not noticeably injured in threshing were sown and the data on the F_2 given in Tables 4 and 5 were obtained for all plants that matured. The F_5 data were obtained on the progeny of 15 F_4 families selected for contrasting plant or head characters. The populations of the F_5 families ranged from 2 to 16 plants each.

The differences between the means of the F_2 and F_5 generations (Table 5) are much smaller than those obtained in the comparison of the F_1 with the F_4, with the exception of length of head and number of spikelets. The trend of the more prominent characters, such as date of heading, height, length of head, and number of spikelets, is toward the emmer parent in the F_5 generation in comparison with the F_2, but in number of awns and in length of awns the F_5 resembles the Aegilops parent more closely than the F_2. The differences between the F_2 and F_5 in height and largest number of awns on the glume are of no mathematical significance.

VARIATION IN THE HYBRID GENERATIONS

The plants of the F_1 generation appeared to possess the uniformity commonly exhibited by F_1 hybrids of intraspecific wheat crosses. The slight variability shown by this generation (Table 4) for the

eight characters studied is probably due to environmental factors. Variability at least as great as that shown by the F_1 should be expressed by all later generations.

In all characters except date of heading, the plants of the F_1 were more uniform than those of the other generations studied. The F_5 generation, grown in a different year from the F_1, showed slightly less variability in heading, but this difference is of no mathematical significance. In all generations date of heading showed the least variability of the eight characters.

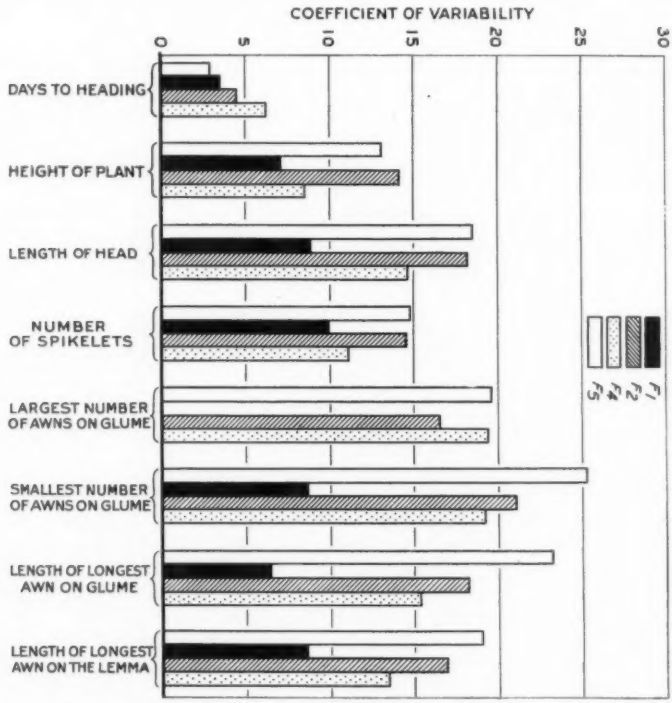

FIGURE 3.—Coefficients of variability of eight characters of four hybrid generations of the cross *Aegilops ovata* × *Triticum dicoccum*

In Figure 3 are shown graphically the coefficients of variability of the eight characters considered in the F_1, F_2, F_4, and F_5. The uniformity of the F_1 as compared to the later generations is evident. The F_5 generation shows the greatest variation in head characters, due undoubtedly to the selection of plants with contrasted characters practiced in preceding generations.

TWO ENTIRE GENERATIONS AND TWO SELECTED FAMILIES OF EACH

In Table 4 comparisons can be made of the characters of the mass F_4 and F_5 generations with those of selected F_4 and F_5 families, respectively. The F_4 families 69 and 66 were selected because of unusual vigor and were grown in the field nursery, while the mass

F_4 generation was grown in the greenhouse. Data on these two families were not included in computing averages shown for the mass F_4. The two F_5 families 19 and 10–3 were grown in the greenhouse and are included in the data for the mass F_5 generation. The two selected F_4 families have equal or lower coefficients of variation, with two exceptions, than the mass F_4 generation for the six characters studied, but the differences are not great, especially in the F_4 family 66.

The F_5 family 19 is known to differ genetically from other families of this generation. The F_4 parent of this line had light-red chaff and the glumes were free from the pubescence common to most plants in all generations of the cross. It was taller than the average of the F_4 and had longer heads and awns. The F_5 family 10–3 was descended from an F_4 plant of low stature with a short head of few spikelets and with short awns on the glumes. These two F_5 families differ significantly from each other in height of plant, length of head, number of spikelets, and length of awns. In addition, the F_5 family 10–3 was constant in having at least one outer glume per spike with a minimum of two awns. The means of both these F_5 families are significantly different from the mass F_5 population in height, length of head, smallest number of awns on the glume, and length of the longest awn on the glume. The longest awn on the lemma of the F_5 family 19 also is longer than that of the mass F_5, while the number of spikelets per head on F_5 10–3 is less than that of the mass F_5. The variation of the mass F_5 generation is greater than that of either of the two selected F_5 families in all the eight characters studied. The two F_5 families 19 and 10–3, however, show in a majority of cases greater variability than the F_1 generation, though considerably less than any other mass generation.

SEGREGATION OF PROMINENT HEAD CHARACTERS

A total of 688 plants of the Aegilops-emmer hybrid have been grown in the F_2 and later generations. The F_2 had a population of 78 individuals; the F_3, 91; the F_4, 380; and the F_5, 139. In the F_4 and F_5 some plants grown in the field are included here that were not included in the tabular data.

Four prominent contrasted characters distinguishing the Aegilops and emmer parents were especially observed in the 688 individuals. These characters were: Glumes scabrous or nonscabrous; glumes black or white (no separation was attempted between white and light-red chaff); the deciduousness of the rachis, that is, the rachis brittle only at a point just below or above the lowest developed spikelet or brittle at each spikelet; and the angle of the awn, whether spreading like the Aegilops or upright like the emmer.

The variations observed in these characters could not be due to back crossing with the emmer or Aegilops parents, or with other wheat or Aegilops. The effect of such back crossing is apparent in case it occurs. Intercrossing between different progeny plants in the F_4 and F_5 generations possibly may have occurred, but in earlier generations the pollinations were controlled. Even if crossing occurred between plants of the F_4, differences in the progeny would result only in case genotypic differences existed.

Glumes Scabrous or Nonscabrous

One F_2 plant grown in 1927 from crosses made in 1926 had nonscabrous glumes, but this individual was completely sterile. The 91 individuals of the F_3 generation had scabrous glumes. In the F_4 generation the 6 individuals of one family were classified as follows: Two very scabrous, 3 lightly scabrous, and 1 as free from spines as the emmer parent. The latter plant gave an F_5 family of 9 individuals all of which were free from spines. The very scabrous and lightly scabrous F_4 plants bred true in the F_5 for these characteristics.

Glumes Black or White

Black glume color, characteristic of the emmer parent, often develops poorly under greenhouse conditions; or, if the heads remain in the greenhouse too long after maturity, fading of the color may result. In some plants of the Aegilops-emmer cross, black color developed only on those glumes where seed set, although in the F_1 it developed regardless of seed setting. All plants with black glumes had pubescent glumes also.

In only one plant in the combined F_2, F_3, and F_4 generations was a fertile white-chaffed form present, this plant being in the F_4. It was both white-chaffed and free of pubescence and gave rise to the white-chaffed family F_5 19, which is shown in Table 4 to differ in plant characters from the mass F_5 generation. One sterile F_2 plant may have been a white-chaffed form, and one family is now being tested for white or reddish-chaffed types, which appeared in the F_5. Despite the failure to develop color in some sterile plants, it appears that the results here agree with those of Kezer and Boyack (*3*), who found linkage between the black color and pubescent chaff of Black Winter emmer.

Deciduousness of the Head

The Aegilops head disarticulates at maturity just below the basal perfect spikelet, the pedicel remaining attached to the lowest perfect spikelet. At this breaking point there is an abscission layer in the rachis, but above this apparently there is none, for the head remains entire. The emmer rachis is brittle at the lower node of each pedicel; in threshing, the head breaks up and the pedicel remains attached to each spikelet of the emmer.

The F_1 hybrid has an abscission layer at the same point of the rachis as the Aegilops and also a layer about equal in development at the lower node of the pedicel just above the basal perfect spikelet. The rachis increases in toughness progressively upward, but an abscission layer is present at each node on its ventral side, while the opposite or dorsal side remains tenacious. Consequently the head of the F_1 is deciduous as a unit just below or just above the lowermost fertile spikelet.

The individuals in the F_2 and later generations have been constant for the F_1 type of deciduousness of head (fig. 2) with the exception of one plant appearing in the F_3 generation in 1928. The head of this plant broke up in the same manner as the head of the emmer and was nearly sterile.

Angle of the Awns to the Rachis

The awns of the Aegilops are spreading, and at maturity may be almost at right angles to the rachis. The awns of the emmer are upright. The angle of the awns with the rachis of the F_1 head is approximately intermediate between the two parents. The later generations showed variations in the spread of the awns. The majority were similar to the F_1, but segregates have appeared with awns as upright as those of the emmer, and more rarely types have occurred that showed a greater spread of awns than is found in the F_1. Figure 4 shows two F_4 types illustrating the semispreading and upright awn forms. The upright-awn type bred true for this character in an F_4 family of 16 individuals, while the semispreading-awn type gave in the F_5 generation 13 semispreading to 2 upright awns.

Germination, Mortality, and Fertility in Five Hybrid Generations

Lethal factors operating at the time of germination, during the vegetative development of the plant, at gametogenesis, and at fertilization may be postulated for a true-breeding hybrid. Table 6 presents data obtained on germination, seedling mortality, and fertility in five generations of the Aegilops-emmer hybrids.

Table 6.—*Germination of hybrid seeds, mortality of seedlings, and fertility in five generations of the cross Aegilops ovata × Triticum dicoccum*

Hybrid generation	Seeds sown	Seeds germinating normally	Seedlings not maturing [a]	Flowers examined	Seeds set in flowers examined	Fertility	Matured plants completely sterile	Range in fertility of individual plants
	Number	*Per cent*	*Per cent*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
F_1	88	89	0	5,877	95	2	40	0-26
F_2	82	96	10	1,828	760	42	20	0-86
F_3	195	66	33	2,115	1,057	50	20	0-83
F_4	63	78	24	2,686	877	33	9	0-89
F_5	114	90	4	2,570	907	35	10	0-88

[a] Includes all seedlings reaching the 3-leaf stage from both normal and abnormal germination.

Eighty-eight F_1, or immediate hybrid seeds, were sown in the greenhouse in 1926, and 89 per cent germinated normally. Of 63 F_4 seeds sown on the same day and under the same cultural conditions as the F_1 seeds, 78 per cent germinated. Of the 82 F_2 seeds sown in 1926, 96 per cent germinated, and of the 114 F_5 seeds given the same greenhouse conditions as the F_2 seeds that year, 90 per cent germinated. The 195 F_3 seeds sown in 1924 showed a germination of only 66 per cent, which is poor for greenhouse conditions.

Theoretically, lethal factors, if present in matured seed, should find expression most frequently in seed produced by the F_1 plants, the F_2 seed; but in this generation the highest percentage of germination was obtained. In Figure 5 are shown kernels of the F_1, F_2, and F_5 generations. The F_1 kernels are typical of the seed obtained in crossing, and the F_2 and F_5 kernels are typical of the seed set in the majority of the plants. Kernels smaller than those shown, and occasionally shriveled, have occurred in the segregating generations, but the majority of the seeds obtained have been larger in size than those of the F_1, the original hybrid seed. (Fig. 6.)

The F_1 seed produced four seedlings which were classed as abnormal, and all died; the F_3 seed produced 18 abnormals, 11 of which died; and the F_4 seed produced 14 abnormal seedlings, 5 of which died.

FIGURE 4.—Two types of awns in the F_4 of *Aegilops ovata* × *Triticum dicoccum:* A, Semi spreading; B, upright. Both heads deciduous at the base. (About two-thirds natural size)

No data on number of abnormal seedlings were obtained for the F_2 and F_5. Abnormal seedlings were those in which the coleoptile failed to develop normally. The pots that showed no plants two to three

weeks after sowing were examined, and the abnormal seedlings were commonly found to be just below the surface of the ground, with the coleoptile curled. In the F_3 lot, four seeds produced radicles but no

FIGURE 5.—Kernel types of parents, hybrids, and back crosses: A, *Aegilops ovata* × F_1 (sesquihybrid); B, F_4 × *A. ovata;* C, F_4 × *Triticum dicoccum* (Black Winter emmer); D, F_3 seeds (borne by F_4 plants); E, F_2 seeds (borne by F_1 plants); F, F_1 hybrid (*A. ovata* × *T. dicoccum*); G, *A. ovata;* H, *T. dicoccum.* × 1½

coleoptiles, and one seed gave a coleoptile but no radicle. One F_4 seed also produced a coleoptile but no radicle. The extremely abnormal individuals all died. The seven abnormal seedlings of the F_3

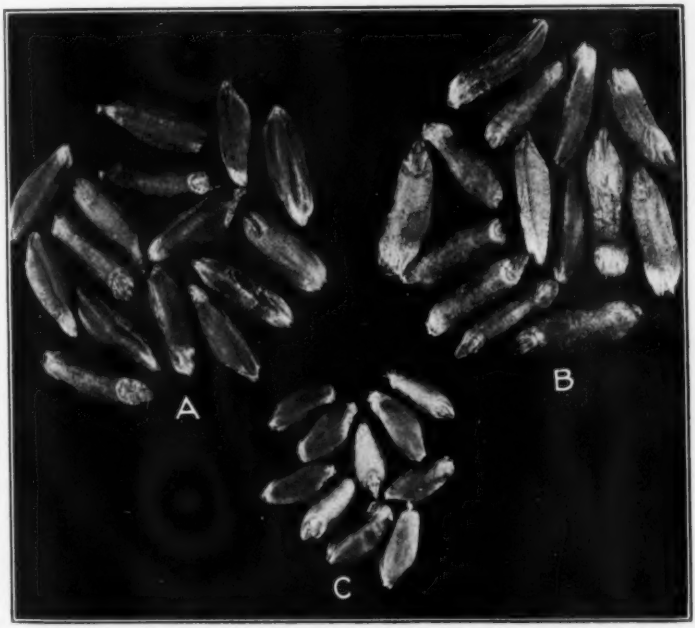

FIGURE 6.—Kernel types of two generations of *Aegilops ovata* × *Triticum dicoccum* and *T. vulgare:* A, Mass F_3 seed (borne on F_1 heads); B, F_3 seed (borne on one F_4 head); C, *T. vulgare* (Canadian Red). Plants are found in the F_2 and later generations with some seeds larger and some smaller than those produced by the F_1 plant. × 2

and the nine of the F_4 that lived developed into plants differing in no noticeable morphological characters from those of the normal plant.

The F_1 seed was borne usually by the Aegilops parent as a result of pollination with emmer pollen. The F_2 seed was borne on the F_1

plant, which has narrower outer glumes than the average plant of the later generations. All seeds sown were removed by hand from the glumes, which persistently retain the seeds. The Aegilops head was dismembered to obtain the F_1 kernels, but in the F_1 and later generations the kernels were removed from the heads by prying the glumes apart with small forceps. Injury to the germ is known to have occurred at times, but the heads were desired so specimens. The tension on the F_2 kernels due to the narrower glumes was not so great as it was in the majority of the plants of the later generations, and injury probably occurred less often to F_2 than to other seed. It is believed that much of the difference in germination percentages and seedling abnormality was due to mechanical seed injury.

All the 78 F_1 seedlings resulting from normal germination produced mature plants. Eleven of the F_1 seedlings were removed to a greenhouse where the daily light period was artificially increased by electric lights. These plants matured earlier than those grown without artificial light and were entirely sterile. The data on these plants are not considered in this paper.

Seedling mortality ranging from 4 to 33 per cent occurred in all generations later than the F_1. The F_4 seedlings grown the same year as the F_1 showed 24 per cent mortality. In the F_2 and F_5 generations grown in 1927 the mortality rates were 10 and 4 per cent, respectively. The 33 per cent mortality in the F_3 generation was at least partly seasonal.

The F_1 generation on the whole showed greater vigor than later generations. Certain families in the F_4 and F_5 were outstanding in vigor, while others were notably weak, requiring careful cultural treatment for maturation.

The fertility percentage (see definition in Description of Characters, p. 664) in the F_1 was 2 per cent; in the F_2, 42 per cent; in the F_3, 50 per cent; in the F_4, 33 per cent; and in the F_5, 35 per cent. Forty per cent of the F_1 plants were completely sterile, while but 9 per cent of the F_4 plants grown the same year were sterile. In the F_2 generation, 20 per cent of the plants were completely sterile, as compared to a 10 per cent sterility in the F_5 in 1927. One F_1 plant showed 26 per cent self-fertility, but in all later generations there were individuals over 80 per cent fertile. No special attempt was made to breed highly fertile strains, but the F_5 family with the highest fertility contained 13 individuals ranging in fertility from 24 to 88 per cent. The average fertility of this family was 48 per cent.

The high sterility of the F_1 Aegilops-emmer hybrid is in part accounted for by the failure of the anthers to develop sufficiently for rupture of the anther sac to occur at the normal time of anthesis. In those heads where dehiscence occurred, it was never complete in all flowers. A microscopic examination of pollen from F_1 plants showed that only 17.8 per cent of the grains were well developed, as compared to 84.5 per cent in one F_4 plant.

In the plants of the later generations anthesis was more normal than in the F_1, but as shown in Table 6, fully sterile individuals were present and usually these had poor anther development. Plants in which the anthers dehisce normally, but which set few or no seed, have been observed.

CROSSING THE HYBRIDS WITH PARENTAL AND RELATED FORMS

In crosses of closely related plants the F_1 and later generations usually hybridize readily with either parent. Distant crosses, however, may give an F_1 which is practically or entirely self-sterile and shows only a slight degree of fertility when crossed with one or both parents. The writers have examined fully 1,000 F_1 wheat-rye hybrids, and with one exception these were self-sterile. The F_1 wheat-rye hybrid, however, back crosses to a limited extent, both naturally and artificially, with wheat and apparently to a less extent with rye.

The Aegilops-emmer hybrid has been crossed with both parents as well as with *Triticum vulgare*, with the results shown in Table 7. Individuals of the F_1, F_3, and F_4 generations were used in these crossing experiments. The data obtained on the F_3 plants were practically the same as those obtained on the F_4, and the data for these generations are combined (as F_4) in Table 7.

TABLE 7.—*Hybridization of individuals of the F_1 and later generations of the cross Aegilops ovata × Triticum dicoccum with parental and other related forms, and the number of hybrids matured*

Female parent	Male parent	Flowers pollinated	Seed set		Hybrid plants matured
		Number	*Number*	*Per cent*	*Number*
F_1 A. ovata × T. dicoccum	A. ovata	42	2	5	2
Do	T. vulgare	26	5	19	2
F_4 A. ovata × T. dicoccum	A. ovata	56	2	4	1
Do	T. dicoccum	244	42	17	22
Do	T. vulgare	56	18	32	10
A. ovata	F_1 A. ovata × T. dicoccum	103	24	23	0
T. vulgare	do	49	2	4	0
A. ovata	F_4 A. ovata × T. dicoccum	204	62	30	0
T. dicoccum	do	549	46	8	0
T. vulgare	do	512	10	2	2
T. durum	do	212	6	3	0

The gametes, both male and female, of the F_1 hybrid were about as effective in producing sesquihybrid seed when back crossed with the Aegilops parent as were the male and female gametes of the F_4 plants. The percentage of seed set in the back cross F_1 × Aegilops was 5 per cent and in the F_4 × Aegilops, 4 per cent. The percentage of seed set in the Aegilops × F_1 was 23 per cent and in the Aegilops × F_4 30 per cent. Only well-developed anthers were selected for pollinating, and as these occurred but rarely in the F_1 plant, the apparent effectiveness of the F_1 as a pollinizer is perhaps exceptional rather than normal.

As the F_1 hybrid had completed its flowering before the emmer parent had begun to blossom, no crosses could be made between them. Between the F_4 and the emmer parent (*Triticum dicoccum*), however, and between both the F_1 and F_4 and *T. vulgare* (Purplestraw and Nebraska No. 28) reciprocal crosses were successfully made, giving the following percentages of seed set (Table 7): $F_4 \times$ *T. dicoccum*, 17 per cent, and *T. dicoccum* × F_4, only 8 per cent; $F_1 \times$ *T. vulgare*, 19 per cent, and *T. vulgare* × F_1, only 4 per cent; $F_4 \times$ *T. vulgare*, 32 per cent, and *T. vulgare* × F_4, only 2 per cent.

The number of flowers pollinated was not large enough to warrant definite conclusions, but it appears that the pollen of the F_1 or of the F_4 is more effective in fertilizing the Aegilops ovule than is the Aegi-

lops pollen in fertilizing the F_1 or F_4 ovule. With *T. vulgare* as the female, however, the pollen of the F_1 or F_4 generations rarely effected fertilization, but *T. vulgare* pollen was fairly successful in fertilizing the F_1 or F_4 ovule. If it is assumed that the hereditary combinations are the same in the reciprocal matings it is probable that different compatibility of the stigma or ovary to the germination of the pollen and penetration of tube exists in the reciprocal matings.

Decidedly different results were obtained in the germination of the hybrid seed, depending on whether the F_1 and F_4 plants were used as the male or as the female parent in matings with the Aegilops and emmer parents and with *Triticum vulgare*. Of the 24 seeds obtained in the mating Aegilops $\times F_1$, none germinated. The 2 seeds resulting from the mating *T. vulgare* $\times F_1$ also failed to germinate. In the reciprocal matings, however, the 2 seeds obtained in the cross $F_1 \times$ Aegilops germinated and gave sesquihybrids. The mating $F_1 \times$ *T. vulgare* gave 5 seeds, 2 of which germinated, and the plants matured. The 46 sesquihybrid seeds obtained in the back cross emmer $\times F_4$ showed no germination, but 22 of the 42 seeds obtained in the reciprocal mating germinated, and the plants matured. The 6 seeds borne on *T. durum* as a result of pollination with F_4 plants failed to germinate. Of a total of 150 seeds obtained through fertilizations with pollen of F_1 and F_4 individuals, only 2 germinated and produced plants; while from the 69 seeds obtained from reciprocal fertilizations, 37 plants were produced.

It was found at the time of threshing that the seeds obtained in the crosses when the Aegilops-emmer hybrid plants were the pollen parents were poorer in endosperm and germ development than those obtained in the reciprocal crosses. The poor development of a seed resulting from a cross of the Aegilops with F_1 pollen and the more normal appearance of the seed obtained from pollinating an F_4 plant with Aegilops pollen are shown in Figure 4.

The reason for the inequality in the development and germination of the reciprocal hybrid seed is not evident. The two hybrid plants matured in the cross of *Triticum vulgare* with an F_4 Aegilops-emmer individual differed in no measurable characters from the plants obtained in the reciprocal cross, indicating that in fertilization no selective assortment of pollen grains by the female plant had occurred.

SUMMARY AND CONCLUSIONS

The investigations reported in this paper deal with the phenotypic expression of definite characters in five generations of the cross *A. ovata* $\times$ *T. dicoccum* (Black Winter emmer). A comparison of the means of eight plant or head characters of the F_1 generation with the F_4 generation grown the same year showed significant differences in six of the eight characters. The variability of the F_1 generation was decidedly less than that of the F_4 in all eight characters.

The means of the plant and head characters of the F_2 generation as compared to the F_5, also grown the same season, showed less pronounced differences, but in five of the eight characters studied the differences probably are significant. The F_5 generation was the most variable of all generations in the head characters studied. Two selected F_5 families, however, showed low variation, and the means of these two families for the eight characters studied showed signifi-

cant differences when compared with the mass F_5 generation and with each other.

In the F_4 and F_5 generations, families differing in glume color and glume surface were isolated, and these bred true. Other differences in head characters were apparent.

The F_1 plant had an average self-fertility of approximately 2 per cent as against 33 to 50 per cent for the later generations. Completely sterile plants occurred in all generations.

From the results obtained in the present study it appears that, although a form intermediate in general appearance to the two parents is maintained in all generations of Aegilops-emmer hybrids, the breeding behavior shows decidedly more variation than is expected in a fixed species. The chromosome complement of the plants appears not fully balanced, and the gametes formed seem not identical in constitution. Consequently sterility and variability result. No entirely true-breeding, fully fertile strain has been isolated from this cross.

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INFECTION EXPERIMENTS WITH THE COTTON ROOT-ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM[1]

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INTRODUCTION

The pathogenic nature of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, has been demonstrated during recent years by various investigators. Successful inoculations of cotton plants have been reported by King (*1*),[2] King and Loomis (*2*, *3*), Peltier, King, and Samson (*5*), Taubenhaus and Killough (*7*), and by Taubenhaus, Dana, Ezekiel, Bach, and Lusk (*6*).

Although the pathogenicity of the fungus and its facultative behavior seem to be well established now, it was not until quite recently that investigators were able to obtain any appreciable amount of infection in large, well-developed plants, especially when pure cultures were used as the source of inoculum. No greenhouse inoculations involving pure cultures have been reported, but King and Loomis (*2*, *3*) have been able to communicate the disease to cotton plants, growing presumably in the open in large tanks or drums, by placing quart-jar cultures of the fungus in holes around the roots of the plants.

In order to gain further information on the parasitism of this fungus, the writers extended these investigations in Texas during the season of 1928–29. Inoculations were made with cotton plants maintained at a constant soil moisture in the greenhouse and with those grown under field conditions. At the time of inoculation the plants in the greenhouse varied in age from seedlings 9 weeks old to plants approaching maturity, while most of those used in the field experiments were well developed and flowering. Various sources of the fungus inoculum were employed, such as sections of infected alfalfa and cotton roots, sclerotia, pure cultures of the fungus, and portions of conidial mats.

The greenhouse work and a portion of the field studies were conducted at the United States San Antonio Field Station, San Antonio, Tex., and the other field inoculations were made at the United States Cotton Breeding Field Station, Greenville, Tex. These experiments and observations are reported in this paper.

GREENHOUSE INOCULATIONS AND TECHNIC

The plants used for the greenhouse inoculations were grown in 5-gallon glazed pots, and the soil, designated as Houston clay loam, was collected from an area considered to be free of root rot. During growth a constant soil moisture was maintained in each pot by subirriga-

[1] Received for publication Apr. 29, 1931; issued November, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 691.

tion through small holes in the sides near the bottom and by weighing at necessary intervals. To facilitate subirrigation, 18 pounds of gravel was added to each pot, sufficient to fill it slightly above the holes on the sides; and the soil, which was added later, was sifted through a ¾-inch mesh screen, placed on a mixing board and worked until quite uniform. The soil was then sampled and the exact moisture content determined. Owing to the large number of pots involved (56), five different mixings of soil were required, and the exact moisture content was determined for each separate mixing. All the pots were finally filled with the soil to within about 4 inches of the top, the quantity varying in the different groups from 11.5 to 12.2 kgm. on a dry basis. They were seeded to Lone Star cotton on January 21 and thinned to six plants per pot on March 8.

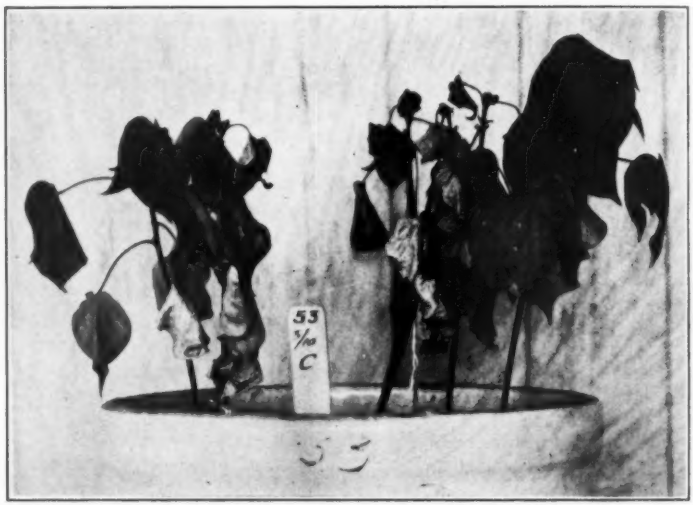

FIGURE 1.—Lone Star cotton plants showing infection 17 days after inoculation with a culture of *Phymatotrichum omnivorum*

The first series of inoculations was made on March 10, and at this time the moisture content in the group of pots used was adjusted to 25 per cent of the dry weight of the soil. During April, May, June, and July, inoculations were made also in other groups of pots having the same moisture content as the first group.

The inoculum consisted of 4-inch and 5-inch sections of alfalfa roots, portions of fresh conidial mats, pure cultures of the fungus grown on sterilized dead cotton roots in 250 c. c. Erlenmeyer flasks, and sclerotia that had been produced in laboratory cultures. During the later stages of the experiments, cultures originating from sclerotia found at Greenville, Tex., in the soil of a field infested with root rot were also used. The sections of infected alfalfa roots were collected from plants that were still alive and possessed active mycelium; the pure cultures employed varied in age from 11 to 35 days; and the sclerotia used had formed in type cultures 9, 37, and 51 days old, respectively.

An attempt was made to use relatively the same quantity of inoculum per plant, but this was difficult to accomplish with the sclerotia, owing to the conglomerate masses encountered when the culture bottles were emptied. The sclerotia were inserted in moistened holes around the roots of each plant in the pots, with the exception of pots Nos. 6 and 7 which received approximately 40 sclerotia each, placed in a single moistened hole in the soil near the center of the pots. The procedure with the pure cultures, conidial mats, and infected alfalfa roots was similar to that described above, a sixth aliquot being inserted near the root system of each plant 4 or 5 inches beneath the soil surface. After the soil and inoculum had been firmly pressed around the plants, the moisture content was adjusted to 25 per cent of the dry-soil weight.

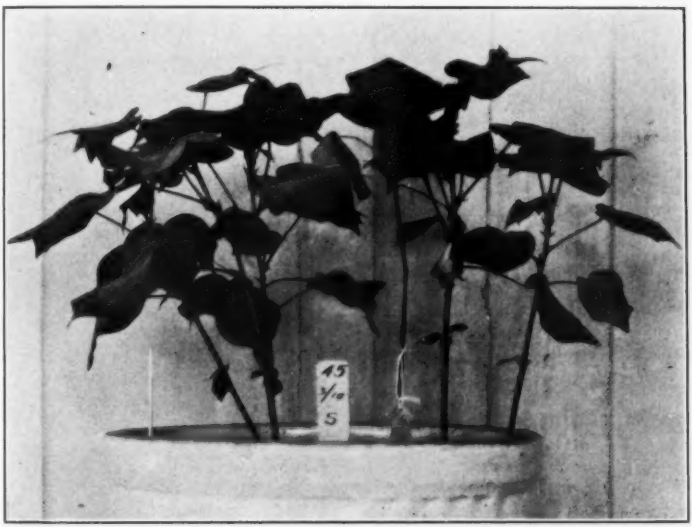

FIGURE 2.—Lone Star cotton plants showing infection 17 days after inoculation with sclerotia of *Phymatotrichum omnivorum* obtained from laboratory cultures

After the inoculations were made the plants were carefully examined every two or three days until September 21. As soon as wilting occurred, they were tagged and the date recorded. Beginning July 24, the pots in which no killing had been observed up to that time were watered, but the maintenance of a constant soil moisture was discontinued. The data for these inoculations are given in Table 1. Some of the pots containing plants inoculated with sclerotia and cultures during March and April are shown in Figures 1, 2, and 3.

The data presented in Table 1 show that the plants inoculated on March 10—pots 49 to 56, inclusive—with a type culture 11 days old produced infection rather uniformly, the minimum period of incubation being 17 days. An exception was noted, however, in the case of pots 54 and 55, which, although reinoculated with the same type culture, but 20 days old, failed to reveal symptoms of the disease up to and including September 21, the date when observations were discontinued.

The roots of some of these plants were infected, however, when examined on October 10. Other plants, as those in pot 11, were somewhat erratic in behavior. Notwithstanding the fact that they were inoculated on two different dates with the same type culture, 9 and 20 days old, respectively, the plants did not show wilting, although the roots of two were infected with the fungus at the close of the experiment.

With few exceptions, the disease developed on the plants inoculated with sclerotia that had formed in laboratory cultures 9, 37, and 51 days old. In most cases the time required for initial infection in these plants varied, with apparently no correlation between age of culture and virulence.

The plants inoculated with infected alfalfa roots on March 10 and again on June 24 showed only relatively slight infection in four of the pots, yet when the roots were examined on October 10 it was found that several were infected and that a few had died since September 21.

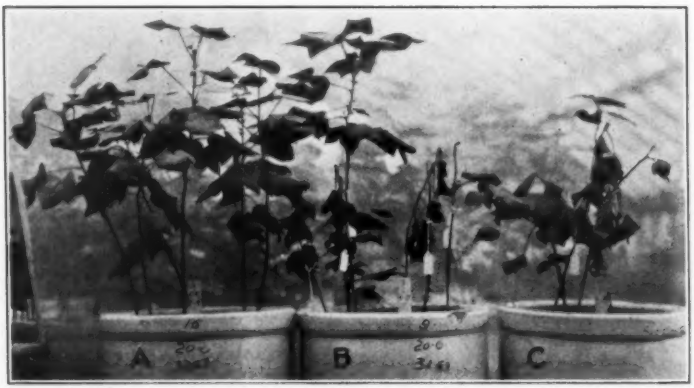

FIGURE 3.—Cotton plants killed by inoculating with sclerotia and cultures of *Phymatotrichum omnivorum*: A, Uninoculated plants; B, plants inoculated with sclerotia; C, plants inoculated with culture.

No infection was obtained from the conidial mat inoculations, the plants remaining healthy during the entire growth period, with no evidence of the fungus on the roots at the conclusion of the experiment. The plants used as controls likewise remained healthy during the course of the experiment.

In as much as the inoculations reported herein primarily embody attempts to demonstrate the facultative behavior of the fungus rather than to emphasize differences in virulence, no special effort has been made to differentiate between primary and secondary infection in the pot experiments. Since all the plants in a pot (six in number) were inoculated, it is of course possible that the activity of one or two separate parts of the inoculum may have been responsible for the infection of all plants in the group, especially when the interval following initial infection was of appreciable duration. In some of the pots, however, the inoculation of each plant resulted in primary infection, as shown by the fact that all wilted on the same day or at very short intervals.

TABLE 1.— *Record of cotton plants grown in greenhouse at the United States field station, San Antonio, Tex., and inoculated with Phymatotrichum omnivorum during the period March to July, 1929*

Date of inoculation and inoculum	Age of culture	Pot No.	Date of wilting and time elapsed from date of inoculation for—												Minimum time for infection
			Plant No. 1		Plant No. 2		Plant No. 3		Plant No. 4		Plant No. 5		Plant No. 6		
March 10:	*Days*		*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Days*
Infected roots [a]		33													65
Do		34	Aug. 28	65	Sept. 5	73									
Do		35													14
Do		36	July 8	14											
Do		37													57
Do		[b] 38	Aug. 20	57											
Do		[b] 39													66
Do		[b] 40	Aug. 29	66											
Sclerotia [c]	37	41	June 19	101	June 20	102	July 5	117	July 30	142	Aug. 23	166			101
Do	37	[b] 42													42
Do	37	43	Apr. 21	42	June 4	86									31
Do	37	44	Apr. 10	31	June 3	85	June 20	102	July 8[d]	120					
Do	37	44							Aug. 21[e]						17
Do	37	45	Mar. 27	17	Apr. 6	27	Apr. 8	29	June 10	92					
Do	37	46													27
Do	37	47	Apr. 6	27	Apr. 12	33	July 31	143							
Do	37	[b] 48													20
B–1 culture [f]	11	49	Mar. 30[d]	20	Mar. 30[d]	20	Mar. 30[d]	20	Mar. 30[d]	20	Mar. 30	20	Mar. 30	20	
Do	11	49	June 15[e]		June 24[e]										20
Do	11	50	Mar. 30	20	Apr. 6	27	June 3	85	July 5	117					36
Do	11	51	Apr. 15	36	May 8	59	May 8	59	May 11	62	May 22	73			48
Do	11	52	Apr. 27	48	...do	59	May 24	75	May 27	78	June 3	85	Sept. 13[d]	187	17
Do	11	53	Mar. 27	17	Mar. 27	17	Mar. 27	17	Mar. 27	17	Mar. 29	19			
Do	11	54													
Do	20	[g] 54													
Do	11	55													
Do	20	[g] 55													
Do	11	56	Mar. 27[d]	17	Mar. 30	20	Mar. 31	21	Mar. 31	21	Apr. 2	23	Apr. 2	23	17
Do	11	56	July 8[e]												

[a] Alfalfa roots. Plants inoculated on Mar. 10 and reinoculated on June 24.
[b] Plants watered but not maintained at a constant moisture after July 24.
[c] Obtained from B–1 culture.
[d] Plant later revived.
[e] Date of second wilting.
[f] Isolated from plot B–1, U. S. Cotton Breeding Field Station, Greenville, Tex., July 17, 1928.
[g] Plants reinoculated June 24.

TABLE 1.—*Record of cotton plants grown in greenhouse at the United States field station, San Antonio, Tex., and inoculated with Phymatotrichum omnivorum during the period March to July, 1929*—Continued

Date of inoculation and inoculum	Age of culture	Pot No. 1	Date of wilting and time elapsed from date of inoculation for—												Minimum time for infection
			Plant No. 1		Plant No. 2		Plant No. 3		Plant No. 4		Plant No. 5		Plant No. 6		
	Days		*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Date*	*Days*	*Days*
Apr. 19:															
Sclerotia [c]	9	9	May 7	18	May 7	18	May 20	31	June 10	52	June 10	52	May 20	31	18
Do	9	10	May 27	38	June 10	52	June 20	62							38
B–1 culture [f]	9	11													
Do	20	[d] 11													
Do	9	12	May 3 [d]												
Do	20	[d] 12			July 1	7	July 1	7	July 11	17					7
May 31:															
Sclerotia [c]	51	6	July 1	31	July 5	35									31
Do	51	7													
July 20:															
D–7 culture [h]	35	1													
Do	35	2	Aug. 8	19	Sept. 6	48	Sept. 13	55							19
Do	35	3	Aug. 7	18	Aug. 20	31	Sept. 6	48							18
Do	35	4													

[c] Obtained from B–1 culture.
[d] Plant later revived.
[f] Isolated from plot B–1, U. S. Cotton Breeding Field Station, Greenville, Tex., July 17, 1928.
[h] Obtained from sclerotia found in the soil at Greenville, Tex., May 24, 1929.

On October 10 the greenhouse experiments were terminated and the number of dead and living plants in each pot was noted. Notes were also made of the plants on whose roots no Phymatotrichum mycelium could be seen and those on which the mycelium was found. These observations are recorded in Table 2.

TABLE 2.—*Condition of cotton plants at the United States field station, San Antonio, Tex., at close of experiment, October 10, 1929*

Inoculum [a]	Pot No.	Plants with uninfected roots	Plants with infected roots		Inoculum [a]	Pot No.	Plants with uninfected roots	Plants with infected roots	
			Alive	Dead				Alive	Dead
		Number	*Number*	*Number*			*Number*	*Number*	*Number*
Infected roots	33	0	6	0	B–1 culture	55	3	3	0
Do	[b] 34	2	3	2	Do	54	4	2	0
Do	35	1	4	1	Do	56	0	0	6
Do	36	1	4	1	Check	13	6	0	0
Do	37	2	3	1	Do	14	6	0	0
Do	38	0	5	1	Do	15	6	0	0
Do	39	6	0	0	Do	16	6	0	0
Do	40	0	5	1	Conidial mats	17	6	0	0
Sclerotia	6	0	1	5	Do	18	6	0	0
Do	7	6	0	0	Do	19	6	0	0
Do	9	0	0	6	Do	20	6	0	0
Do	10	0	1	5	Do	21	6	0	0
Do	41	0	1	5	Do	22	6	0	0
Do	42	6	0	0	Do	23	6	0	0
Do	43	0	4	2	Do	24	6	0	0
Do	44	0	1	5	Check	25	6	0	0
Do	45	0	2	4	Do	26	6	0	0
Do	46	1	4	1	Do	27	6	0	0
Do	47	0	4	2	Do	28	6	0	0
Do	48	6	0	0	Do	29	6	0	0
Check	[c] 8	3	0	0	Do	30	6	0	0
B–1 culture	11	4	2	0	Do	31	6	0	0
Do	12	0	1	5	Do	32	6	0	0
Do	49	0	2	4	D–7 culture	1	2	3	1
Do	50	0	2	4	Do	2	2	1	3
Do	51	0	0	6	Do	3	1	2	3
Do	52	0	0	6	Do	4	6	0	0
Do	53	0	1	5	Check	5	6	0	0

[a] For dates of inoculations and other data see Table 1.
[b] Seven plants in this pot at beginning of experiment.
[c] Three plants in this pot at beginning of experiment.

RECOVERY OF THE FUNGUS IN CULTURE

The fungus was recovered in culture on July 8 from infected roots in pots 6 and 12, and on August 12 from those in pot 3. This was accomplished by following the usual method of isolating the fungus from infected cotton plants. Small sections of the infected roots were washed thoroughly in tap and distilled water to remove all traces of the soil, surface sterilized from 30 to 45 seconds in a 1 : 1,000 solution of mercuric chloride, dipped for a brief period in 50 per cent alcohol, and transferred to Petri dishes containing corn-meal agar. Growth of the fungus started after three days in the dishes, and was identified microscopically as *Phymatotrichum omnivorum* on the above-mentioned dates.

FIELD INOCULATIONS AT GREENVILLE, TEX.

Inoculations were made with plants in the field at the United States Cotton Breeding Field Station, Greenville, Tex., in July, August, and September, 1928. On July 17 in an area free from root rot on plot

C–8, 32 plants were inoculated with 6-inch and 7-inch sections of infected roots from freshly wilted cotton plants. Twenty consecutive plants in one row were inoculated, while the remaining 12 plants, in two rows of 6 plants each, had 4 feet of row space with healthy plants intervening between each 2 plants inoculated. Twelve plants in other rows were reserved as checks. The inoculum was placed in moistened holes 4 or 5 inches below the surface, near the plant roots, and was covered with moist soil. The inoculated plants were watered twice weekly thereafter. One plant in the consecutive inoculations developed root rot on July 27, and another in the other area became infected on August 4. In both cases the fungus was recovered in culture by the method previously mentioned. The plants reserved as checks remained healthy.

On August 14, immediately south of the laboratory, in an area free from root rot, two groups of 16 plants each were inoculated with infected cotton roots. These roots were divided into two lots, according to the type of visible mycelium, and designated as "active white" or "ochraceous buff." One type of inoculum was used in each group of plants. In this experiment 8 plants were reserved as checks. The dates of examination and results are given in Table 3.

TABLE 3.—*Results of field inoculation of cotton plants with Phymatotrichum omnivorum at Greenville, Tex., August 14, 1928*

Plants inoculated	Inoculum	Type of mycelium	Plants infected with Phymatotrichum						
			Sept. 5	Sept. 13	Sept. 17	Sept. 24	Sept. 28	Oct. 18	Total
Number			*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
16	Infected roots	Active white	2	0	0	2	4	4	12
16	do	Ochraceous buff	4	0	2	0	0	0	6
8 checks	None		0	0	0	0	0	0	0

Although both types of mycelium in different stages of growth produced the disease, there were indications, in this experiment at least, that the fungus on recently attacked roots is perhaps more virulent than that on roots which have been infected for a longer period. On the former the fungus growth usually shows as a fine white mycelium, whereas on the latter the mycelium is generally coarse and brown to ochraceous buff in color.

SOIL-CORE INOCULATIONS

Previous attempts to transmit the disease to healthy cotton plants by means of cylindrical cores of soil collected from the margin of active zones of infection in alfalfa fields have been reported by King and Loomis (*2*). In their experiments soil cores having spore mats on the surface were inserted in the soil adjacent to plants growing in tanks and drums. As to the results obtained by this method of inoculation the authors comment as follows:

> None of the cotton plants died as a result of this treatment, but when the cores of soil were dissected in December live mycelium was still in evidence on partially decayed pieces of alfalfa root. There was no evidence, however, of the mycelium being extended into the soil surrounding the cores, and the greater part of the mycelium was dead and brittle.

In order to obtain more information regarding the dissemination of the fungus and to determine whether there is any likelihood of its being transmitted in soil from infested areas by such agencies as cultivating implements, balled nursery stock, or drainage water, several inoculations involving transfers of soil from fields infested with the disease were made during the summer of 1928. The soil was removed in cylindrical cores on August 14 from the periphery of an active zone of infection at the Greenville station by means of a specially modified piece of cast-iron pipe, measuring roughly 7 inches in diameter and about 10 inches in length. As soon as the soil cores were collected they were placed in large jars to avoid shattering or breaking, wrapped in moist burlap, and transferred immediately to a zone free from root rot, where they were used to replace soil cores removed from between well-developed plants.

Although 22 inoculations were made, this method failed to establish new infections during the remainder of the season or the year following. However, the season of 1929 was extremely dry, and therefore, no doubt, was unfavorable for the development of the disease. This should be taken into consideration in interpreting the negative results obtained.

Moreover, since these experiments were conducted a sclerotium stage of the fungus has been observed in laboratory cultures by King and Loomis (*3*), and in nature by the senior author (*4*). Therefore, it is possible that the failure to obtain infection by the soil-transfer method described may have been due in part to the fact that sclerotia were not present in the soil cores and that the fungus existed only in the mycelial state when the inoculations were made. While it is reasonable to assume that the fungus may have been entirely vegetative at this period—August 14—yet as late as July 12 at Greenville and July 19 at San Antonio, during the season of 1929, viable sclerotia were recovered from infested cotton fields at depths corresponding, in some cases, to those at which soil cores were taken.

It appears, therefore, that further investigation is needed to determine the possibility of conveying the disease to new locations by any practice involving the transfer of soil from infested areas. Such investigation is especially desirable in view of the discovery of the occurrence of the sclerotia in nature, as they undoubtedly constitute an important means of carrying the fungus in a viable condition through an extended period of inactivity.

FIELD INOCULATIONS AT SAN ANTONIO, TEX.

Eleven plants, grown from cuttings in the greenhouse and later transferred to the field on April 19, were inoculated with pure cultures of the fungus on June 17. This particular culture, B–1, previously referred to in this paper, had been isolated from infected cotton plants the preceding year and had since been cultivated continuously in the laboratory on corn-meal agar. The inoculations were made in a manner similar to that described earlier in this paper, that is, by inserting the contents of flask cultures of the fungus a few inches below the surface in a moistened area around the plant roots. Additional inoculations were made in the same way on July 19, and at this time another culture, D–7, which originated from sclerotia found in the soil of an infested field at Greenville, was included.

Twelve additional plants, similarly propagated, were inoculated on this date, 4 with culture B–1 and 8 with culture D–7. At each inoculation about 5 feet of green plants were left in the rows between the inoculated plants, some of which were tagged to serve as controls.

On July 1, three of the plants inoculated with B–1 culture on June 17 developed root rot and later communicated the disease to adjacent healthy plants. The disease also developed in one of the plants inoculated with the D–7 culture on July 19, and was later recovered in culture on August 12 from the infected roots.

SUMMARY

Studies bearing upon the pathogenicity of the cotton root-rot fungus, *Phymatotrichum omnivorum*, were made during the season of 1928–29 at two localities in Texas.

Successful inoculations are reported with cotton plants grown in the greenhouse at a constant soil moisture and with others grown under field conditions.

The inoculum employed consisted of pure cultures of the fungus, cultures originating from the sclerotia found in the soil, sclerotia obtained in laboratory cultures, and conidial mats. With the exception of the conidial mats, the inoculum used produced infection in the plants both in the greenhouse and in the field, and in some cases caused the death of the host.

Although many of the pure-culture inoculations, both vegetative and sclerotial, resulted in visible infection of plants in the pot experiments, some were erratic in behavior, showing great variation in the minimum time required for initial infection.

Cultures varying in age, especially those in which sclerotia were present, did not show perceptible differences in virulence when the plants were inoculated, both young and old cultures exhibiting considerable variation with respect to the minimum time required for infection.

Although many of the plants inoculated in the greenhouse failed to reveal visible symptoms of infection during growth, the roots of several were found to be infected with the fungus at the close of the experiment.

The fungus was recovered in culture from plants on whose roots the types of inoculum produced infection, and was later identified microscopically.

Several plants inoculated with infected roots in the field at Greenville during the summer of 1928 developed the disease. There were some indications in these experiments that the fungus on freshly infected roots was more virulent and killed a greater number of plants than that on roots in which the infection was of longer duration.

Efforts to transmit the disease to new areas at Greenville by means of soil cores obtained from an active zone of infection were unsuccessful. A probable explanation is offered for the failures reported and the need for further investigation is emphasized.

Cotton plants grown in the greenhouse in 1929 from cuttings and later transferred to the field at San Antonio were successfully inoculated with pure cultures of the fungus isolated in 1928. Cultures obtained from sclerotia found in nature were employed also and their pathogenicity established with cotton plants similarly grown.

The results herein reported corroborate the work of other investigators in showing that the destructive disease of cotton known as root rot in Texas and other Southwestern States, is caused by the fungus *Phymatotrichum omnivorum*. The fact that the fungus, after subsisting for long periods in the laboratory on culture media, may infect healthy plants growing under both greenhouse and field conditions, is indicative of its facultative behavior.

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A PRELIMINARY REPORT ON THE POISONOUS EFFECTS OF BITTER RUBBER WEED (ACTINEA ODORATA) ON SHEEP[1]

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INTRODUCTION

Since 1925 a small annual weed, known in western Texas as "bitterweed," has become an increasingly serious menace as a sheep-poisoning plant. This plant, *Actinea odorata*, is related to the Colorado rubber weed, *A. richardsoni*, and the two plants produce very similar effects on sheep.

The attention of the United States Department of Agriculture was first called to the injurious effects of *Actinea odorata* in December, 1925, by W. H. Drew, of Eldorado, Okla. This town is situated in the extreme southwestern corner of the State near the Texas line. Mr. Drew sent to the department a sample of a plant which he said was killing his sheep. He did not state the location of the range in question but said that in his 25 years' experience as a sheepman this was the first time that he had had trouble from this plant. His description of the symptoms shown by the sick sheep correspond very well with those which more recently have been produced experimentally. The specimen of the plant which he sent was identified by S. F. Blake, of the Bureau of Plant Industry, as *A. odorata*.

Attention has since been called to *Actinea odorata* by investigators at the Texas Agricultural Experiment Station. Cory[2] in 1926 stated that the plant is "reported to be called 'lemonillo' * * * we sometimes referred to it as 'bitterweed.'" He stated further that the plant causes the loss of sheep in the latter part of the winter, that under favorable conditions it may bloom in almost any month of the year, that it is a heavy producer of seed, and that proper moisture conditions may be followed by a good growth of seedlings.

Cory[3] in 1926 said also that "bitterweed" causes sheep to become sick and that the plant is eaten only when feed is scarce and no other small green growth is available. He described the general effects of the plant on sheep.

As a result of preliminary feeding experiiments made at the Texas Agricultural Experiment Station, Conner[4] in 1929 stated:

> Obnoxious as is this weed, some sheep will eat it during the late winter when other green weeds or grass are not available, or at least are very scarce. Altnough repeated force-feeding tests have not been absolutely satisfactory, they strongly suggest the toxic character of this plant when consumed in large quantities because of the hemorrhagic gastroenteritis found at autopsy.

Schmidt[5] in 1930 stated that sheep eat the bitterweed, especially in the early spring when they are hungry for something green. He

[1] Received for publication May 5, 1931; issued November, 1931.

[2] CORY, V. L. RANGE OF VEGETATION AND THE FEEDING HABITS OF CATTLE, SHEEP, AND GOATS. Sheep and Goat Raisers' Mag. 7(2): 13–18. 1926.

[3] CORY, V. L. RANGES HARBOR POISON PLANTS. ONLY HUNGER DRIVES LIVESTOCK TO EAT HARMFUL WEEDS. Cattleman 13(5): 19–21, illus. 1926.

[4] CONNER, A. B. BITTER WEED (ACTINEA ODORATO[A]). Tex. Agr. Expt. Sta. Ann. Rpt. 42: 141. 1929.

[5] SCHMIDT, H. STOMACH WORMS AND BITTER WEED. Sheep and Goat Raisers' Mag. 11(4):29. 1930.

also stated that sheep have been poisoned by being force fed 2 or 3 pounds of the plant and that the Texas station has no remedy for the poisoned animals.

The National Wool Grower for January, 1931,[6] called attention to the losses due to bitterweed. It stated that "several hundred sections are covered with it * * *. It's spreading each year, an octopus of the range."

Cory,[7] who for several years has been closely associated with the range problems of western Texas, in 1931 called attention to the distribution and the increasing abundance of this plant in that section and emphasized the relationship between the abundance of the bitterweed and the heavy grazing of the range. On January 9, 1931, he wrote to this bureau as follows:

> *Actinea odorata* has become more abundant and more widely spread this season than heretofore. We have recent reports from the Pecos High Bridge country on the southwest and from the Abilene country on the northeast. The trouble due to this plant started earlier than usual this season and is about at its height at the present time. It appears to constitute a serious menace to the sheep industry over a large part of the Edwards Plateau.

Again, on January 26, 1931, he wrote:

> This plant blooms at any time of the year when moisture conditions are favorable, and it seeds heavily. Ordinarily livestock do not eat this plant. However, in the winter months, when it is very abundant, and the only green herbaceous material available, sheep eat it. They apparently form a liking for it, and after some days they get down. * * * After late fall or early winter rains this plant carpets the ground and appears almost as a carpet of fescue grass. There are plants in bloom now, and they may be found in bloom all other months of the year. Ordinarily they cause trouble from January 15 to February 15 here, but the trouble began the middle of December this season.

Although this plant is ordinarily a menace only to sheep, a few correspondents have stated that goats and cattle are poisoned by it.

In view of the foregoing reports and the evident menace of the plant to stock raising, the Bureau of Animal Industry undertook the investigations the results of which are here presented. The author likewise personally examined affected ranges in Texas and obtained first-hand information from livestock owners whose animals had fallen victims to the destructive plant.

The increase in losses from *Actinea odorata* poisoning, to which thousands of sheep succumbed early in 1931, is probably an indirect result of overgrazing the range. This kills out the valuable forage and gives a chance for the less desirable and obnoxious plants to get a foothold and to increase in number. Animals usually eat poisonous plants because of a lack of the proper kinds of feed.

DESCRIPTION AND DISTRIBUTION OF THE PLANT

Actinea odorata, a member of the Compositae, is an annual weed that grows to a height of from 6 inches to 2 feet and has a branching stem. The leaves are alternate and have very narrow or linear divisions. The more or less erect branches terminate in yellow flower heads which are one-fifth to one-third of an inch in diameter. Figure 1 shows the general appearance of this plant.

[6] ANONYMOUS. WEST TEXAS NEWS [BITTERWEED, A RANGE PEST]. Natl. Wool Grower 21(1): 47. 1931.
[7] CORY, V. L. BITTERWEED. Sheep and Goat Raisers' Mag. 11(7): 1926–27. 1931.

Although the term "lemonillo" has been applied to *Actinea odorata* by a few botanists, it is known in western Texas as bitterweed and in places in New Mexico as rubber weed. The name bitterweed,

FIGURE 1.—General appearance of bitter rubber weed (*Actinea odorata*). This specimen was about 1 foot in height

however, is much more generally applied to *Helenium tenuifolium*, and the term "rubber weed" is used for *A. richardsoni*. In order to distinguish *A. odorata* from these other plants the name bitter rubber weed[8] is suggested.

[8] "Bitter rubber weed" was suggested by M. W. Talbot, in charge of weed investigations, Division of Botany, Bureau of Plant Industry.

Actinea odorata is distributed from southwestern Kansas and central Texas westward across southern New Mexico and southern Arizona to southeastern California, as shown in Figure 2,[9] and thence southward into Mexico. Its range extends from a little above sea level to an altitude of about 4,000 feet. It is not found in the mountains and, as a rule, it does not occur in areas of high humidity.

Although bitter rubber weed is fairly widely distributed, with one exception the records of animals poisoned by it are all from the Edwards Plateau region of western Texas and east of the Pecos River.

PLAN OF THE EXPERIMENTS

Through the kindness of Edwin Jungherr, of the Texas Agricultural Experiment Station at Sonora, a liberal supply of *Actinea odorata* was obtained in the spring of 1930. This material, consisting of leaves, stems, roots, and flowers, was dried and shipped to the bureau's experiment station at Salina, Utah.

During the summer of 1930 the plant was fed experimentally to 11 sheep. All the dosages were computed in terms of the green plant, 75 per cent being allowed for the loss in weight by the plant in drying. Although no drying tests have been made with *Actinea odorata*, a considerable number with *A. richardsoni* and other plants have shown 75 per cent, by weight, to be a fair estimate of the amount such plants lose in drying. In all the feedings the entire plant, including leaves, stems, flowers, and roots, was used, and in all but one the plants were finely ground, moistened with water, and fed by means of a veterinary balling gun. While the sheep were being used in these experiments they were given a liberal supply of alfalfa hay. The feeding experiments are summarized in Table 1.

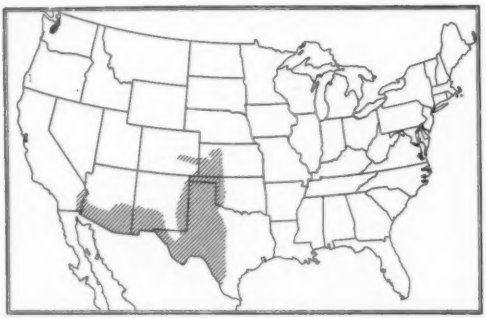

FIGURE 2.—General distribution of *Actinea odorata* in the United States

In an attempt to produce acute sickness, the ground plant was given in a single feeding in seven of the experiments. In these feedings the quantity of dry plant varied from 0.075 to 0.325 per cent of the animal's weight, or from 0.3 to 1.3 per cent of its equivalent of green plant. In the other four experiments the plant was given in small daily feedings for a considerable period. Three of the sheep were given the moistened plant by means of a balling gun, and, in order to distribute it over the day, each day's dose was, as a rule, divided into three parts, one part being given in the morning, one about noon, and one in the late afternoon. One sheep was fed the dry plant mixed with hay. These cases are termed "chronic."

[9] W. W. Eggleston, of the Bureau of Plant Industry, furnished the information regarding the distribution of this plant.

TABLE 1.—*Summary of feeding experiments with Actinea odorata (leaves, stems, flowers, and roots)*

Sheep No.	Weight of animal	Date or period of feeding	Method of feeding	Dose as percentage of animal's weight [a]		Result
				Total	Average per day	
	Pounds					
1150	67	June 17–July 7	Balling gun	5.25	0.25	Very sick.
1152	75	June 20	do	[b] 1.00		Slight symptoms.
1158	81	June 25	do	[b] 1.20		Do.
1161	94	June 27	do	[b] 1.30		Death.
1162	80	July 2	do	[b] .75		Not sick.
1165	76	July 9	do	[b] .50		Do.
1170	93	July 14–Sept. 25	do	7.40	.10	Symptoms.
1185	115	Aug. 6	do	[b] .30		Not sick.
1186	130	Aug. 9–Sept. 7	With hay	7.24	.24	Do. [c]
1187	82	Aug. 9–Sept. 25	Balling gun	4.80	.10	Sick.
1196	102	Aug. 16	do	[b] .40		Not sick.

[a] Estimated as green plant. [b] Given in 1 feeding. [c] Loss in weight the only effect observed.

RESULTS OF THE EXPERIMENTS

SYMPTOMS

The symptoms produced in both the acute and chronic cases of poisoning were very similar. They were principally salivation, nausea, vomiting, depression, and weakness. In the acute cases the pulse was distinctly faster and weaker and the body temperature higher after the feeding than before. In the chronic cases the illness continued for a longer time and the weakness was more pronounced than in the acute cases.

Sheep 1150 is a good example of a chronic case. When this sheep, a yearling ewe, was placed on the experiment June 11, she weighed 67 pounds. She was active, vigorous, and had a very good appetite. After being kept under observation for six days she was given daily, with the balling gun, 12.6 gm. of the dry, finely ground plant. The first day this was divided into two equal doses, one being given at 11.50 a. m. and the other at 2.20 p. m. After the first day each day's dose was divided into three approximately equal parts. These feedings were continued daily through July 7. Computed in terms of green plant, the quantity given each day was equivalent to 0.25 per cent of the weight of the sheep. Each day of the experiment she was given a liberal supply of alfalfa hay. On July 2 she weighed 73.5 pounds.

The first evidence of illness was noticed in the afternoon of July 3, when a foamy liquid was seen about the nostrils. The next morning she was nauseated, there was a quantity of green regurgitated material around the nose and mouth, and she had not eaten all the hay fed the preceding day. At 7.55 p. m. she appeared somewhat weak and walked unsteadily. The symptoms of illness became more pronounced, and by 5 p. m., July 6, she had vomited, her breath smelled strongly of stomach contents, and she was so weak that she preferred to lie down. On the last day on which she was fed the *Actinea odorata*, July 7, she was much nauseated and very weak. Most of the time she was lying down. When on her feet she stood with her back arched and walked with difficulty, and after taking a few steps she

began to tremble noticeably. Her appetite, however, was good. Both the nausea and the weakness became more marked. On July 8 she weighed 62.5 pounds, having lost 11 pounds in six days. On the morning of July 9 she was barely able to get on her feet. Although she had vomited profusely during the night of July 8, she ate hay greedily. During the day she was nauseated and lay down most of the time, and on the morning of July 10, she was unable to stand. She vomited and showed other evidence of nausea for the last time during the night of July 10. She gained strength slowly and at 5 p. m., July 15, she could get on her hind feet but not on her forefeet. When lifted to her feet and held in that position she trembled. She made repeated attempts to get up but was unable to do so until 10 p. m., July 18. At this time she managed to get on her feet with great effort but trembled and had to lie down at once. At 9 a. m., July 19, she got on her feet and stood for a few minutes, although in an awkward manner. Her forelegs were bent at the knee joint, and the feet were drawn back under the body. The forelegs were also bent at the fetlock joints so that she stood almost on the front edges of the hoofs. She was able to stand but a few seconds at a time. Although she gained somewhat in strength she was unable to walk more than a few steps at a time for the next four days. On July 23 she was put into a small pasture where she could be kept under observation. At 1.10 p. m., when disturbed, she got on her feet readily and ran a few steps in an awkward manner. From this time on she slowly gained strength, and the forelegs gradually straightened until on August 6 she stood and walked in a practically normal manner. At this time she was still somewhat weak and thin, and she did not regain her usual activity until about August 19, when she weighed 66 pounds.

This sheep gained in weight up to the day before definite symptoms were noted, but after that she lost flesh rapidly. She was not weighed during the period of most severe illness, but from August 3 until September 15 she gained steadily. The rate of respiration during the first part of the feeding period did not vary to any great extent, but during the latter part of the feeding and up to the time she began to show marked improvement it varied greatly.

On September 26 she was shot. The autopsy was negative, except for a slight enlargement of the lymph glands associated with the stomachs.

TIME BETWEEN FEEDING AND APPEARANCE OF SYMPTOMS

When *Actinea odorata* was fed in a single large dose, cases of an acute type were produced. For these the time from the end of the feeding to the first appearance of symptoms is shown in Table 2.

TABLE 2.—*Time between feeding and appearance of symptoms in animals fed a single large dose of Actinea odorata*

Sheep No.	Dose as percentage of animal's weight [a]	Time between feeding and appearance of symptoms	
		Hours	*Minutes*
1152	1.0	20	25
1158	1.2	6	30
1161	1.3	5	50

[a] Estimated as green plant.

In cases of this kind in which loss of appetite and depression are the first symptoms noted, it is of course impossible to give a definite time for the appearance of the effects. The time when some abnormality in the animal's actions or condition was first observed is taken as the beginning of symptoms. It is realized, of course, that the figures given in Tables 2 and 4 are, in a sense, approximations, because it would be quite impossible accurately to ascertain that an animal was well one minute and ill the next, or vice versa, but it has been thought best to present the actual experimental results as obtained. In the three animals given a single large dose of the plant the time from the feeding to the appearance of symptoms varied from 5 hours and 50 minutes to 20 hours and 26 minutes, and averaged 10 hours and 55 minutes. The larger the dose the sooner the symptoms appeared.

The reports of field cases indicate that sheep are more commonly poisoned by eating small quantities of the plant at frequent intervals for a number of days than by eating large quantities at one time. In order to determine the quantity a sheep must eat daily to be affected four sheep were fed for a considerable period. When small repeated feedings were given it was found that very small daily doses if continued for a sufficiently long time would cause illness. The time required to produce symptoms in these four cases is shown in Table 3.

TABLE 3.—*Time between the beginning of experimental feedings with Actinea odorata and the appearance of symptoms when small daily doses were given*

Sheep No.	Average daily dose as percentage of animal's weight [a]	Time between first feeding and appearance of symptoms
		Days
1150	0.25	17
1170	.10	44
1187	.10	46
1186	[b].24	[c]23

[a] Estimated as green plant. [b] Mixed with hay. [c] Loss in weight the only symptoms shown.

Sheep 1150, which was given daily 0.25 per cent of its weight of the plant, showed symptoms of illness in 17 days, and after 19 days it was very ill. The feeding was continued for 21 days during which the sheep was given a total dose of 5.25 per cent of its own weight. At the close of the feeding it was thought that the animal had probably received a lethal dose. However, it made a partial recovery.

Of two sheep, each given a daily dose of 0.1 per cent, one showed slight but distinct symptoms of poisoning after it was fed for 44 days and the other after 46 days. The feeding of these animals continued at the same daily rate after the symptoms were noticed. One sheep remained in about the same condition, showing evidence of slight poisoning. It was fed a total dose of 7.4 per cent of its own weight in 74 days. The symptoms of the other sheep became more pronounced, and after 48 days of feeding it was distinctly ill.

The fourth sheep, No. 1186, was fed the *Actinea odorata* mixed with hay. Although it did not become distinctly ill, it began to lose

in weight after it had eaten the plant for 23 days. During this time it ate an average daily dose of 0.24 per cent of its own weight.

From these experimental cases it is apparent that a daily dose of 0.1 per cent of the weight of the animal may produce distinct symptoms of poisoning in 44 days and more pronounced illness in 48 days, whereas a daily dose of 0.25 per cent may produce symptoms in 17 days and severe illness in 19 days.

DURATION OF ILLNESS

Inasmuch as the acute and chronic cases differed materially in duration of illness, they are considered separately. The lengths of time during which symptoms were observed in the three acute cases are given in Table 4.

TABLE 4.—*Length of the periods of illness for the three sheep fed single large doses of Actinea odorata*

Sheep No.	Daily dose as percentage of animal's weight [a]	Duration of illness		
		Days	*Hours*	*Minutes*
1152	1.0	3	8	14
1158	1.2	2	16	27
1161	1.3		[b] 12	

[a] Estimated as green plant. [b] Died.

The average duration of the illness in the two sheep that recovered was 3 days and 20 minutes. The sheep that died lived about 12 hours after it became sick.

For the sheep fed *Actinea odorata* daily for a protracted period, the duration of illness has been measured by the time they were sick after the last feeding. Two of the sheep made ill by this method of feeding were shot for autopsies at the end of the feeding period. This leaves but one animal, No. 1150, from which to determine the duration of illness. This sheep was more or less ill for 33 days and at the end of that time she was still thin and had not entirely regained her strength.

The duration of illness in both acute and chronic cases is at best an estimation. There is no sharp line between illness and recovery. In fact there is some question as to the final complete recovery of such cases.

AUTOPSY FINDINGS

An autopsy was made of one animal that died from acute poisoning. The pathological changes were well marked and were evidently due to the action of a severe irritant on the walls of the first and fourth stomachs and of the duodenum. The mucosa of the rumen, especially about the opening of the esophagus, was congested. On the outer surface of this organ, especially in the groove between the first and second stomachs, and along the anterior edge of the spleen there was a mass of coagulated serum. The mucosae of the fourth stomach and of the duodenum were inflamed, and the wall of the fourth stomach was somewhat edematous. Injurious effects were not apparent in the other organs.

Two sheep that had been given daily doses of the plant for 48 and 74 days, respectively, were killed at the end of the feeding period. The results of the autopsies on them were negative. The one animal that was very sick was not killed until its condition had very much improved. The lymph glands near the stomachs in this sheep were somewhat enlarged. Otherwise, nothing abnormal was found when the autopsy was made.

SUMMARY

Actinea odorata, known in western Texas as bitterweed, a plant of the semiarid Southwest, is distributed from southwestern and central Texas to southeastern California and southward into Mexico. On the Edwards Plateau of Texas it seems to have been increasing rapidly in abundance since about 1925 and now appears to be a serious menace to the sheep industry.

Feeding experiments have demonstrated that the plant is toxic to sheep, the principal symptoms produced being salivation, nausea, vomiting, depression, and weakness.

These experiments have shown that if a sheep consumes 1.3 per cent or more of its own weight of the green plant within a short time, fatal results may follow. They have shown also that when a sheep eats as little as 0.1 per cent of its weight of the plant daily it may become ill in about 44 days, and that larger daily doses will produce effects in a correspondingly shorter time.

THE DISTRIBUTION OF STIMULATIVE EFFICIENCY IN THE ULTRA-VIOLET SPECTRUM FOR THE HONEYBEE[1]

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INTRODUCTION

During the last 50 years considerable evidence has gradually accumulated that insects and arthropods in general are sensitive to ultra-violet light. Lubbock (*1, p. 152–168*)[3] was one of the first to experiment in this field, and he found that ants carry their young out of a region illuminated by ultra-violet into one illuminated by light of longer wave lengths. Forel (*3*) confirmed this observation and convinced himself that the seat of this sensitivity is in the eyes and not in the epidermis of the ant's body.

One of the most indefatigable workers on the reactions to light and color among animals was Von Hess, who wrote voluminously of his experiments and theories between 1907 and 1922. Von Hess showed unmistakably that bees and several other arthropods are sensitive to ultra-violet light. In some of his experiments (*5, p. 928*) he confined 20 to 50 bees in a plate-glass box 20 by 10 by 8 centimeters and wrapped the box in a large piece of black paper in such a way that a tunnel about 40 centimeters long was formed, with the box placed near one end. He placed next to a window the end containing the box and made observations through the opposite end. He then put various light filters between the window and one half or the other of the side of the glass box lying next to the window. One of these filters was a Schottschen Schwerstflintglas (abbreviated Sfl.) which transmitted only the so-called visible rays of the spectrum; i. e., waves longer than 400 mμ. He says, in describing the behavior of the bees in one of these experiments,

> Bringe ich vor die eine Hälfte das Sfl., so gehen sie in wenigen Sekunden nach der anderen, ultraviolettreicheren Seite, *auch dann noch, wenn diese durch Vorhalten eines Grauglases* oder mit anderen passenden optischen Hilfsmitteln *für uns wesentlich dunkeler gemacht wird, als die ultraviolettarme.*

He went on further to find out how much more stimulative the unfiltered light was to the bees than the light filtered through the Sfl. filter. He says (*5, p. 928*):

> * * * ich konnte * * * feststellen, dass unter bestimmten Bedingungen an einem bewölkten Tage das durch Sfl. uv.arm gemachte Tageslicht auf die Bienen nicht anders wirkt als wie relativ uv.reiches Tageslicht, dessen Stärke für uns nur etwa 1/8–1/9 des uv.armen ist und unserem Auge neben dem letzteren ziemlich dunkel grau erscheint!

[1] Received for publication Apr. 3, 1931; issued November, 1931.

[2] Professor of biology, Western Maryland College, Westminster, Md. The writer is indebted to James I. Hambleton, in charge of the Division of Bee Culture, Bureau of Entomology, U. S. Department of Agriculture, for the use of facilities and for many courtesies; to Prof. S. O. Mast, of the Johns Hopkins University for much kindly advice; and to Dr. W. W. Coblentz and R. Stair, of the Bureau of Standards, U. S. Department of Commerce, for spectroscopic measurements.

[3] Reference is made by number (italic) to Literature Cited, p. 713.

He says further, parenthetically, that by the use of a mercury-vapor lamp he was able to show that for ants the light from this lamp, very rich in ultra-violet, is as stimulative as ordinary light made 200 times stronger. Of course, by "stronger" Von Hess meant only more brilliant to his own eye, which does not mean necessarily that it really contained more energy, but, nevertheless, if ordinary daylight had to be reduced to one-eighth or one-ninth of its original intensity in order to stimulate bees to the same lower degree as the same light with the ultra-violet filtered out, it is very probable that the ultra-violet is more efficient in its stimulation for bees than is the so-called visible light.

Von Hess investigated, furthermore, how far the spectrum visible to arthropods extends into the ultra-violet. He used for this purpose only certain caterpillars and crustaceans, but found (*5, p. 929*) "dass die Bewegungsrichtung von Raupen und Krebsen noch deutlich beeinflusst wird durch Strahlen von ca. 313 mμ * * *." He found this sensitivity to ultra-violet to be of general distribution among the arthropods, but concluded (*4, p. 199*) that it is due to a fluorescence of the outer parts of the ommatidia whereby the short waves are changed to longer ones, and not to true vision of the ultra-violet.

Tests of a similar nature, involving only the nontrained (instinctive) reactions of the animals in question, were made by Lutz and Richtmyer (*9*) in 1922 on Drosophila, and by Lutz (*8*) in 1923 on various insects which visit flowers, including the honeybee. All these insects reacted definitely to ultra-violet. In Lutz's experiments, for example, he made a box in each end of which was a window consisting of one or another of several light filters. The top of the box was of glass surrounded by an opaque hood within which he could put his head, drawing the free end of the hood around his neck to exclude the light but enabling him to see inside the box. The box was carried to the field, and into it were placed insects caught at flowers. In some of the experiments he used in one end of the box a very dark filter which transmitted almost no light except ultra-violet, and in the other various clear and chromed glasses which transmitted almost no ultra-violet. When he placed a worker honeybee in this box he found that it went from every one of his chromed filters to the filter transmitting ultra-violet, and when at the latter "would not leave it to go to several thicknesses of clear glass," although to the human eye the light coming through the several thicknesses of clear glass was enormously brighter than that coming through the other and was probably of considerably greater energy.

Meanwhile Kühn and Pohl (*7*) in 1921 reported the results of their experiments on the honeybee involving training. These investigators first trained bees to come for food to a narrow trough illuminated by the light of the ultra-violet line 365 mμ of the mercury-vapor spectrum, and then, after the bees were trained, they removed the food and allowed the image of the entire spectrum to fall on a piece of white paper. The bees that now came for food congregated almost exclusively on the region illuminated by the light of the line at 365 mμ. Various checks seemed to show that the distinction was not based on the relative position of this line with respect to the others, nor on its relative brilliance; and Kühn and Pohl conclude, therefore, that light of wave lengths near 365 mμ is not only visible to bees but is

qualitatively distinguished by them from that of other wave lengths of the mercury-vapor spectrum. This fact indicates that the ultra-violet probably appears to the bee as a color distinct from any other color in the mercury-vapor spectrum, and not, as Von Hess postulates, merely as a fluorescence. A continuation of this set of experiments by Kühn (*6*) led to the further conclusion that the spectrum for the bee extends at least to wave length 313 mμ (as Von Hess had found for "Raupen und Krebsen"), and that this wave length is also distinguished from those longer than 400 mμ, but not from wave length 365 mμ.

Furthermore, the experiments of the present writer (*2*) on the relative efficiency for the bee of regions in the so-called visible spectrum showed that at 431 mμ, the lowest wave length attainable with his apparatus at that time, the efficiency is still one-tenth as great as at its maximum in the yellowish-green region at about 553 mμ. The efficiency at 431 mμ is thus relatively considerably greater for the bee than for the human eye—a fact which tends to confirm the evidence that the spectrum visible to the bee extends to considerably shorter wave lengths than does that visible to man.

The results of these various researches show clearly that bees are sensitive to ultra-violet light down to at least wave length 313 mμ, and indicate that the efficiency of this light is fairly high. The next step is to find how efficient various regions in the ultra-violet are in stimulating the bee, and to ascertain more accurately, if possible, how far into this portion of the spectrum the vision of the bee extends. If the efficiency at wave length 431 mμ is 10 per cent as great as at 553 mμ, one wonders if it gradually drops off until the limit of perception is reached or if it again rises to another maximum. It was to throw further light on these questions that the experiments now to be described were performed.

METHOD

The procedure necessary to find the relative stimulative efficiency of different regions of the spectrum by the method chosen in this investigation involves three steps: (1) To find the relative stimulative effect of each region studied, in terms of the intensity of white light necessary to equal the effect of that region in stimulating the bee; (2) to find the relative physical energy emitted by each of these regions; and (3), in each case, to divide the value for the relative effect by the corresponding value for its relative energy, obtaining as a result a series of values representing the relative efficiency (i. e., effect per unit of energy) of the several regions.

The apparatus for carrying out the first step of this procedure is shown in Figure 1. The main box, *a*, was shallow and rectangular, 67 by 26 by 4 centimeters inside, and provided with a hinged lid covered with screen wire. In the ends of the box were three openings, one, *b*, in what will be designated as the left-hand end, and two, *c* and *d*, in the right-hand end. Openings *b* and *c* were each covered by a piece of ground glass, opening *d* by a piece of ground quartz. Opposite opening *b* was a small lamp house, *e*, containing a 15-watt lamp the light from which passed through a small aperture and illuminated the ground-glass screen behind that opening. Opposite opening *c* was

a long lamp house, *f*, having a total length of 140 centimeters and containing a lamp mounted on a support sliding on a track, so that within the limits of the apparatus the lamp could be placed at any desired distance from the opening. The light from this lamp went through a rectangular aperture and illuminated an area 6.5 by 13.0 millimeters on the ground-glass screen covering opening *c*. By the use of a 10-watt lamp for the lower intensities and a 75-watt lamp for the higher, and by placing the lamp at stated distances ranging from 40 to 126.5 centimeters from the ground-glass screen, the screen could be illuminated with light having relative intensities in the proportion of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 129, 258, 387, 516, 645, 774, 903, 1,032, 1,161, and 1,290. (The minimum intensity 10 equals approximately 3.5 meter-candles.) A glass cell, *g*, 3 centimeters thick inside the glass walls, containing a solution of copper sulphate in the proportion of 19 grams per liter, screened out the infra-red and heat rays.

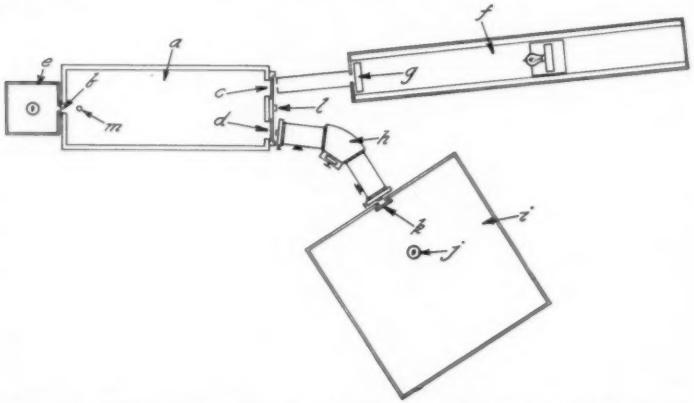

FIGURE 1.—Diagram (plan) of apparatus used to ascertain the intensity of white light necessary to produce stimulative effects upon bees equal to those of the light from selected lines of the mercury-vapor spectrum. (For explanation, see text.)

Opposite opening *d* was placed the telescope end of a spectroscope (Monochromator), *h*, of the constant-deviation type; all its lenses and prisms were of quartz, and opposite the collimator end of this spectroscope was placed a lamp house, *i*, containing a 220-volt, quartz mercury-vapor lamp, *j*, of the horizontal type, run with a current of about 5 amperes (the Cooper-Hewitt "Uviarc"). Light from this lamp passed through the spectroscope *h*, by which any desired line of the mercury-vapor spectrum could be selected and made to illuminate the screen of ground quartz covering opening *d*, forming there an illuminated area of the same size and shape as that formed on the screen covering opening *c*. Lines of the wave lengths 280 mμ, 297 mμ, 302 mμ, 313 mμ, 334 mμ, 365 mμ, 405 mμ, and 436 mμ were used. A glass filter, *k* (Corning Glass Works', "Corex A, red purple"), was placed between the mercury-vapor lamp and the spectroscope in all experiments in which a line of wave length shorter than 405 mμ was used, since this filter cuts off all visible light above wave length

405 mμ, thus excluding any stray visible light that otherwise often finds its way through a spectroscope by reflection from the inside wall of the tube of the instrument.[4]

The lamp in house *e* was turned on or off as desired by means of a small switch, but the lamp in house *f* and the mercury-vapor lamp *j* in house *i* were left on continuously during the course of an experiment, the light coming from them being cut off at will from opening *c* and *d* by means of a shutter, *l*, sliding vertically in a track.

A hole, *m*, in the floor of the large box *a*, permitted the entrance of the bee used in an experiment. The whole apparatus was placed in a dark room maintained constantly at a temperature of about 12° C.

In performing an experiment with this apparatus, a bee at a feeder outside was captured in a small screen-wire cylinder about the size of an ordinary test tube, taken inside the dark room, and fed with sugar sirup (by inverting over the cylinder a small vial containing this liquid, the mouth being covered with a layer of cheesecloth) until it was satiated, then released into the large box *a*, through the hole in the floor near the left-hand end. The feeding of the bee, together with a temperature in the dark room lower than that outside, tended to quiet the bee and prevent it from racing wildly about the box when released into it, and the time taken for feeding allowed the eyes of both the bee and the observer to become somewhat adapted to the darkness.

When the bee entered the box all was dark except the two illuminated areas in openings *c* and *d*. On seeing these the usual reaction of the bee was to walk rapidly toward the right-hand end of the box, and as it approached this end to veer toward one or the other illuminated area, depending, presumably, on which was the more stimulative to it. As soon as it could be ascertained toward which illuminated area the bee was going, the opaque shutter *l* was drawn up, cutting off the light from these areas, and at the same time the lamp in box *e* was turned on. At this, the bee turned around and walked rapidly toward the left-hand end of the box. As soon as it had come back near the starting point the lamp in box *e* was turned off and at the same time the shutter *l* dropped down, at which the bee turned and walked again toward the right-hand end of the box, veering often toward the same illuminated area as before, sometimes toward the other area. This marching and countermarching was continued until the bee had made five trips toward the right-hand end of the box, a record being kept as to which illuminated area it approached on each trip. Then it was recaptured in the wire cylinder, taken into the open and released, and a new bee brought in and treated in the same way. For comparing the stimulative effect of the light of any given line of the mercury-vapor spectrum with any given intensity of white light, from 5 to 20 bees were used, giving a total of from 25 to 100 reactions.

Usually a very low intensity of white light was used in the first test with any spectral line. If the reactions of the bees indicated that this intensity of white light was less stimulative than the spectral light—that is, if less than 50 per cent of the reactions were toward the white—it was raised to a slightly higher intensity, and another

[4] The advisability of using such a filter was suggested to the writer by Prof. A. H. Pfund, of the Johns Hopkins University.

test made. If this showed that the white light was still less stimulative than the spectral light, the intensity of the former was raised again and another test made. This procedure was continued until an intensity of white light was found which definitely induced more responses than did the spectral light.

By finding in this way some intensities of the white light which produced less than 50 per cent of the total responses and some which produced more than 50 per cent, and then by plotting these values and interpolating, an intensity was computed which, theoretically, would have produced exactly 50 per cent of the total responses. The intensity thus computed was regarded as the value of the stimulative effect of the particular spectral line under consideration. In a similar way values were obtained for all the spectral lines used. This procedure accomplished the first step mentioned at the beginning of this section as being necessary in the method here chosen.

The second step, the finding of the relative energy emitted by each spectral line used, was done in the radiometry laboratory of the United States Bureau of Standards by W. W. Coblentz and R. Stair.

There remained then only the third step, the dividing of the value representing each relative effect by that representing each corresponding relative energy, in order to obtain values representing the relative efficiency of each spectral line.

RESULTS

The results of the experiments to find the relative intensities of white light necessary to produce upon the bees the effects of the several spectral lines used are presented in Table 1. It will be observed that for wave length 280 mμ all the reactions of the bees were toward the white light, even at the lowest intensity producible with the apparatus; that is, the bees entirely ignored the spectral light of wave length 280 mμ, and the probability is that they did not see it at all. When light of wave length 297 mμ was compared with white light at an intensity of 10, in the scale of intensities named on page 706, 88 per cent of the reactions were toward the white light, indicating that the bees saw this spectral line, but that it was much less brilliant to them than was the white light even at that very low intensity. For all other wave lengths used, however, intensities of white light were found some of which elicited fewer responses than did the spectral line (less than 50 per cent of the total), same more.

The intensity of white light which would elicit exactly as many responses as the given spectral line is, of course, what is needed. This can easily be ascertained by plotting the percentage going to the white light as an ordinate, with the intensity of that white light as an abscissa, and connecting the points by a curve, as is done in Figure 2 for each wave length used. In making these curves only intensitites were considered for which as many as 50 responses had been observed, since the larger numbers of observations presumably gave the more reliable results, and these intensities furnished sufficient points to establish the curves desired. By examining the graphs, the point at which each of the curves crosses the line representing 50 per cent of the total responses toward the white light can be easily ascertained, and this point for each spectral line gives the relative stimulative

effect of that line in terms of the intensity of white necessary to produce its effect on the bees. These values are given in the second column of Table 2.

TABLE 1.—*A summary of the reactions of bees to different intensities of white light when these intensities were exposed to them simultaneously with light from selected lines of the mercury-vapor spectrum*

Wave length (in millimicrons)	Relative intensity of white	Number of reactions observed	Percentage going to the white	Wave length (in millimicrons)	Relative intensity of white	Number of reactions observed	Percentage going to the white
280	0.10	25	100	334	0.60	100	60
	1.29	25	100		1.00	100	70
297	.10	50	88		3.87	25	100
302	.10	100	48		6.45	25	80
	.30	100	62		9.03	25	88
	.50	100	65		11.62	25	96
313	.10	25	24	365	.20	25	0
	.50	50	48		.30	25	16
	.60	25	20		1.00	25	4
	.70	25	80		1.29	25	20
	.80	25	32		2.58	25	16
	.90	25	32		3.87	100	27
	1.00	50	28		5.16	25	44
	1.29	100	39		6.45	100	40
	2.58	100	53		7.74	25	48
	3.87	100	63		9.03	100	52
	4.40	25	36		10.32	25	68
	5.16	25	40		11.62	100	58
	6.45	100	55		12.90	25	88
	7.74	25	48	405	.10	100	21
	9.03	100	58		.20	100	60
	10.32	25	72		.40	100	59
	12.90	50	74	436	.10	100	40
334	.20	100	38		.20	100	56
	.40	100	50		.40	50	68

TABLE 2.—*The relative stimulative effect of the light from each spectral line used, as obtained from Figure 2, together with the measured energy of each spectral line (where measurable), and the calculated efficiency of each*

Wave length (in millimicrons)	Relative stimulative effect (=S)	Relative energy (=E)	Relative stimulative efficiency (=S/E×5,206.7)
280	None.		0
297	(a)	1.3	?
302	0.1	7.6	68.5
313	2.3	55.9	214.2
334	.4	5.3	393.0
365	8.6	100.0	447.8
405	.2	51.8	20.1
436	.15	78.1	10.0

[a] Less than 0.1.

The relative energy emitted by each of the spectral lines used (except by the line at 280 mμ, which was too small to be measured) was, as has been stated, measured by a thermopile and galvanometer at the United States Bureau of Standards. The results of these measurements are given in the third column of Table 2.

When the values for relative stimulative effect are divided by the corresponding values for relative energy and the dividend multiplied by 5,206.7 (in order to bring the value for 436 mμ to 10.0) the resulting values for stimulative efficiency are those presented in the last column of Table 2. It will be observed that the efficiency is low at 302 mμ, the shortest wave length for which complete data were obtained, and

that it rises gradually through 313 mμ and 334 mμ to a maximum at 365 mμ, from which it decreases suddenly again toward 405 mμ and 436 mμ.

The relation of these values to each other and to those previously found (*2*) for the longer wave lengths, as well as to those for the human eye, are strikingly shown in Figure 3. In the previous work, just referred to, the efficiency at wave length 431 mμ, the lowest attainable with the apparatus used at that time, was found to be 10.11 per cent as high as the maximum at 553 mμ. In order to join the curve formed by the values obtained in the present investigation as closely as practicable to that formed by the values found for the longer wave lengths, the efficiency at 436 mμ was given an arbitrary value of 10.0 and the

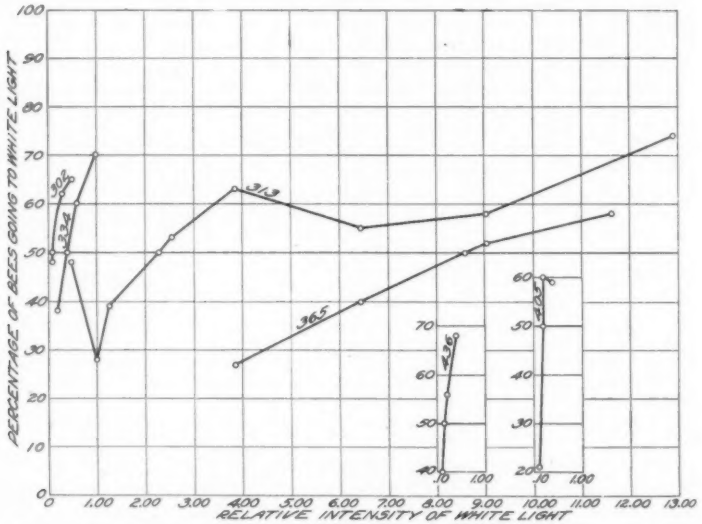

FIGURE 2.—Curves plotted from data given in Table 1, in order to find by graphic interpolation the relative intensities of white light needed to produce stimulative effects upon bees equal to those of the light from selected lines in the ultra-violet portion of the mercury-vapor spectrum. The point at which each curve crosses the line representing 50 per cent of the responses toward the white locates the relative intensity of light which should be required to produce a magnitude of response equal to that produced by the light from the spectral lines indicated

others were related to it. The complete curve shows that the efficiency at 365 mμ, the maximum for the ultra-violet, is nearly four and one-half times as great as that for 553 mμ, the maximum in the so-called visible portion of the spectrum. The relative values derived for efficiency of the two maxima, expressed for comparison on the same scale, are 447.8 and 100.0.

DISCUSSION

The results of this investigation, while somewhat to be expected from the work of Von Hess and Kühn, are nevertheless a little startling. For no other animal has it been shown, so far as the writer has found, that the curve of the distribution of stimulative efficiency has more than one maximum. Furthermore, the fact that the maximum

in the ultra-violet is so much greater than that in the so-called visible spectrum again raises the old question as to the significance of floral colors in attracting insects. It is not the writer's purpose to review the various controversies on this question, but simply to indicate the possible contribution of this investigation.

The nature of the visual stimulation which a bee receives from looking at a flower in sunlight obviously depends on three factors: (1) The nature of the sunlight, i. e., the length of its spectrum and the distribution of energy therein; (2) the nature of the reflection by the flower, i. e., the wave lengths reflected and the intensity of

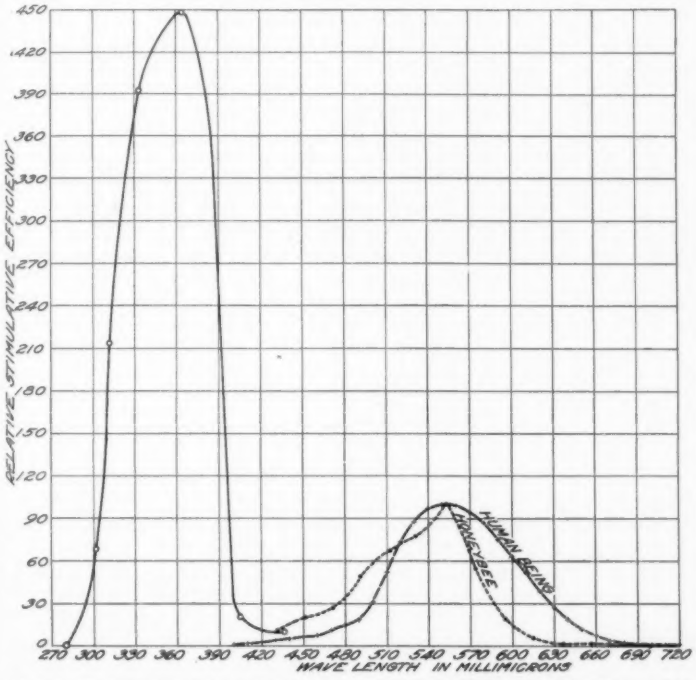

FIGURE 3.—Distribution of stimulative efficiency in the ultra-violet spectrum for honeybees (solid line), together with a reproduction of the corresponding curve for the visual spectrum as found in a previous investigation (dotted line) and with the corresponding curve for the human being

light of each wave length relative to the intensity of each present in the sunlight; and (3) the nature of the bee's photoreceptors, i. e., the lengths of waves to which it is sensitive and the relative sensitivity for the different wave lengths.

The spectrum of the sunlight reaching the earth's surface extends from about wave length 290 mμ to comparatively long infra-red rays, but for our present interest we may consider only the portion from 290 mμ to 700 mμ. If this portion be divided at wave length 450 mμ into an ultra-violet and a visual section, the former will contain, roughly, about one-fifth of the total energy of this portion, and the latter about four-fifths (*11, p. 418, Table 551*).

The nature of the reflection of flowers has been taken up in late years by Richtmyer (*10*) and Lutz (*8*). Richtmyer shows the curves for ultra-violet reflection for a number of flowers, and Lutz shows photographs of flowers taken through a filter transmitting only ultra-violet. Lutz concludes (*8, p. 240*) that "* * * perhaps about 30 per cent of conspicuous flowers are distinctly ultraviolet" as compared with the fact that "perhaps about 50 per cent of the conspicuous flowers are strongly blue" and "* * * nearly 80 per cent of such flowers are spectroscopically strongly red." For an animal which can see ultra-violet as a distinct color, it appears, then, that this color must have a really significant place in the sensation which the animal gets from looking at flowers in nature.

Is the honeybee such an animal? Can it see ultra-violet as a distinct color, and do so efficiently enough to make these short waves significant at all in its vision? The answer seems to be in the affirmative. The work of Kühn (*6*) gives convincing evidence that to the bee ultra-violet is qualitatively different from other colors, and the evidence presented in the present paper leaves no reasonable doubt as to the efficiency of these short waves in stimulating the bee. In fact, if the energy in the ultra-violet section of sunlight amounts to one-fifth of the total energy in the portion of the sun's spectrum visible to the bee, and if this ultra-violet energy is four and one-half times as efficient in its stimulation as the rest of the energy, it may be concluded that the actual visual stimulation given to a bee by the ultra-violet portion of sunlight is nearly as great as that given by the so-called visual portion, and therefore that in the stimulation given by those "30 per cent of conspicuous flowers" which, according to Lutz are "distinctly ultra-violet," this color forms practically as conspicuous a part of the bee's sensation as does any other color.

SUMMARY

The results of various researches have shown clearly that honeybees are sensitive to ultra-violet light and have indicated that the efficiency of this light is fairly high.

In the present investigation of the relative efficiency, in stimulating reactions of honeybees, of the light from certain lines of the ultra-violet spectrum, a mercury-vapor lamp, of quartz, was used as the source of the radiation studied. Light from this lamp was passed through a spectroscope (Monochromator) provided with lenses and prisms of quartz, and from the resulting spectrum ultra-violet light of eight different wave lengths was tested for its effect on the bees.

The bees experimented upon were admitted, one at a time, into one end of a shallow, oblong box, in which their actions could be observed. At the opposite end of the box were two small screens alike in shape and size, one of ground quartz and the other of ground glass. Ultra-violet light, transmitted by the spectroscope, of one of the eight wave lengths chosen was thrown upon the quartz screen, at the same time that the screen of ground glass was illuminated by white light. The reaction of the bee under observation was recorded, as to which of the two screens it approached, the box being in darkness except for the two controlled illuminations. By a special light at the opposite end of the box the bee was attracted back to its starting point. After five such trips the bee was released and another was substituted.

The illuminations of the ground glass with white light were so controlled that they were always of known relative intensities, and so chosen and compared as to determine what would be the relative intensities of white light producing stimulative effects upon the bees equal to those of the several illuminations with ultra-violet light.

Finally, the relative energy of each illumination with ultra-violet light was separately determined. By dividing the values of the several stimulative effects by the corresponding relative values for energy, the relative efficiency—i. e., the relative stimulative effect per unit of energy—of the ultra-violet light of each wave length concerned was derived. These results show that the efficiency is highest at wave length 365 mμ, and that the spectrum extends at least down to wave length 297 mμ, but not to 280 mμ. When the curve representing the distribution of stimulative efficiency in the ultra-violet spectrum is joined to that for the so-called visible spectrum, as found by the writer in a previous investigation, there are shown to be two maxima, one in the yellow-green, at wave length 553 mμ, and the other in the ultra-violet, at 365 mμ, the latter being about four and one-half times as great as the former.

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THE DECOMPOSITION OF GREEN MANURES GROWN ON A SOIL AND TURNED UNDER COMPARED TO THE DECOMPOSITION OF GREEN MANURES ADDED TO A FALLOW SOIL[1]

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INTRODUCTION

In studying the effect of green manuring upon the microbiological processes taking place in the soil the practice frequently has been to add to a relatively small volume of soil a certain quantity of either green or dry plant material and incubate it under laboratory or greenhouse conditions. In a recent publication (*8*)[3] the writers reported the results of adding a heavy application of green rye and green vetch to two acid and neutralized soils in greenhouse benches. The material was grown on other benches to avoid any effect on the soil that might be due to the growth of the plants. While this is in line with the usual laboratory procedure it is open to a rather serious criticism. It has been known for some time that the growth of higher plants has a profound influence upon the soil flora. Various explanations have been offered for this, such as the effects of the mineral or organic secretions of the roots, the dying and decomposition of the outer cells of the roots, the solvent action of the roots or of the carbon dioxide produced by them upon mineral matter, the production of toxins and the pseudosymbiosis of plants with certain groups of bacteria. Starkey (*9, p. 325–326*) made an exhaustive search of the literature upon this subject and came to the conclusion "that the development of higher plants results in modification of the soil population and that marked increases in the abundance of certain microorganisms frequently occur." In some cases the plate counts of the soil organisms may be increased, but on the whole the data on the plate counts are rather conflicting and not so definite as those obtained in studying the numbers and activities of certain organisms or groups of organisms.

It is conceivable that adding green manures to a fallow soil might not, for other reasons, be comparable to turning under a green-manure crop into a soil which had produced it. For instance, if rye is grown and turned under it is generally understood that no nitrogen or mineral salts are added to the soil which were not extracted from that soil by the growing plant. Therefore, treating a fallow soil with rye tops, or with the whole plant, would be, in fact, adding to that soil fertilizing elements which had been taken out of another soil. Nevertheless, this method has usually been employed in studying the decomposition of green manures. Some investigators have grown green manures and turned them into the soil on which they were grown, but in these cases only a few data were obtained on nitrate

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[2] The authors wish to thank Charles Thom, principal mycologist, for suggestions and interest in the work, and Daniel Ready, assistant scientific aid, for nitrate and moisture determination.
[3] Reference is made by number (italic) to Literature Cited, p. 731.

accumulation or the number of microorganisms. These determinations were made after long intervals and give no picture of the intermediate effects of the treatment.

Therefore, it seemed highly desirable to compare some of the microbiological effects produced by the decomposition of green manures in a soil on which they had grown with the effects produced by the decomposition of the tops of a green manure grown on another soil and added to a fallow soil.

OUTLINE OF EXPERIMENTS AND METHODS

The soil used was Leonardtown clay loam which was taken from an uncultivated field near Leonardtown, Md. It was brought to the greenhouse in bags, and after being thoroughly mixed was placed in benches 3 feet square and 10 inches deep. Duplicate plots received enough ground limestone to bring the soil to the neutral point. Distilled water was added daily to keep the soil at the optimum moisture content.

The first eight plots of this soil were used in the previous study (*8*), whereas the second set of four plots was planted at that time to rye or vetch. These green manures were turned under, but no study of their decomposition was made. At the completion of the earlier work, all plots except Nos. 1 and 2 were treated with muriate of potash at the rate of 200 pounds per acre and superphosphate at the rate of 500 pounds per acre.

For the experiments reported in this paper another crop of rye and vetch was planted on plots 9 to 12, inclusive. These were turned under when the rye was about 1 foot high, the vetch being lower and more or less matted upon the soil. At the same time a like amount of green rye and green vetch tops, which had been grown in another greenhouse especially for this purpose, was added to the first set of plots. Table 1 gives the weights and nitrogen content of the green manures as well as the plan of the experiment. It should be noted that the quantity of roots in the soil where the green manure was grown was not determined; also, that the amounts of dry material and of nitrogen in the tops added to the fallow soil were not the same as grew on the soil, although the green weights of the tops were the same for the respective plots. However, in three of the four cases more nitrogen was added to the fallow soil than was present in the tops of the cropped soil. This compensated somewhat for the presence of the roots in the latter.

TABLE 1.—*Weights of rye and vetch tops added to fallow soil, or grown on a soil and turned under, and their nitrogen equivalents*

Plot No.	Treatment	Green weight per acre	Dry weight	Equivalent dry weight per acre	Nitrogen content	Nitrogen per acre
		Pounds	*Per cent*	*Pounds*	*Per cent*	*Pounds*
3	Rye tops added	18,200	12.4	2,257	3.47	78
4	do.[a]	20,600	12.4	2,554	3.47	89
9	Rye grown	18,200	12.4	2,257	3.16	71
10	do.[a]	20,600	9.6	1,978	3.26	65
7	Vetch tops added	9,200	13.8	1,270	4.26	54
8	do.[a]	5,500	13.8	759	4.26	32
11	Vetch grown	9,200	19.6	1,803	2.81	51
12	do.[a]	5,500	38.2	2,101	2.69	57

[a] Limed series.

Soil samples were taken with a tube to a depth of 6 inches, six cores being used for a composite sample. These were taken just before and 2, 4, 7, 14, 21, 35, and 56 days after green manuring. Nitrate nitrogen was determined by the zinc-copper couple method (*7*), as follows: To 250 gm. of moist soil, 100 ml. of aluminum cream and 400 ml. of distilled water were added and the suspension shaken frequently over a period of 20 minutes. The suspension was then filtered, the first 15 or 20 ml. being discarded. The filtrate was divided into two aliquots and the ammonia dispelled by boiling with 5 gm. MgO–NaCl mixture. The original volumes were then made up with distilled water, the zinc-copper coils added, and the resulting ammonia distilled into boric acid. Titration was made with standard acid equivalent to 0.5 mgm. N per milliliter and the results calculated to the dry-soil basis.

The number of microorganisms was determined by plating the appropriate dilution (1 to 500,000 or 1 to 1,000,000) on soil-extract agar (*8*) and incubating for three weeks at 28° C. The number of fungi was likewise determined by the plate method. In this case the dilutions were 1 to 5,000 or 1 to 10,000 and the medium was acid dextrose-nitrate agar (*8*). The incubation period was for three days at 28°.

The amount of carbon dioxide evolved from the surface of the soil was determined as recently described (*2*). Essentially, this method is as follows: A zinc box 3 by 8 inches and 3 inches deep with a small hole in each end is placed bottom side up and pressed about 1 inch into the soil. Air is drawn over the soil by a vacuum pump and through KOH. A blank determination for carbon dioxide is run on the air of the room and this is subtracted from the total amount of carbon dioxide found, to give the amount evolved from the soil. The amount of air in both cases is regulated by flow meters.

EFFECT OF TREATMENTS ON THE NITRATE CONTENT OF THE SOIL

The accumulation of nitrates after green manuring has been studied extensively and the literature has been adequately reviewed (*4*, *5*, *6*). Because of the frequent use of nitrate accumulation for indicating decomposition of green manures it was necessary to make nitrate determinations in order to compare the results obtained in this work with those obtained by others.

It will be recalled that the soils receiving rye or vetch tops in this experiment had had two previous applications of green manure and that the other soils had previously grown a green-manure crop which was turned under. The effect of this treatment and the effect of more than a year's incubation in the greenhouse are plainly seen in Figure 1. The nitrate content of the unplanted soil, 87 parts per million for the unlimed and 130 parts per million for the limed soil, was not only considerably higher than was anticipated but was decidedly higher than is encountered under ordinary conditions. Nevertheless it was thought advisable to continue the experiment. In contrast to the fallow soil, the unlimed soil on which rye had grown had only 2 parts per million of nitrates before the green manure was turned under, and the limed soil had 17 parts per million. This difference between cropped and fallow soil has often been observed

by other workers (*9*), the explanation usually being that the difference is due to the absorption of the nitrates by the growing plants. But it has also been said that certain crops inhibit the accumulation of nitrates in soil, i. e., they depress nitrification, and that this is especially true of nonlegumes. On the other hand, legumes are said to be more favorable to the accumulation of nitrates. The data obtained in the present work bear out these statements, as Figure 2

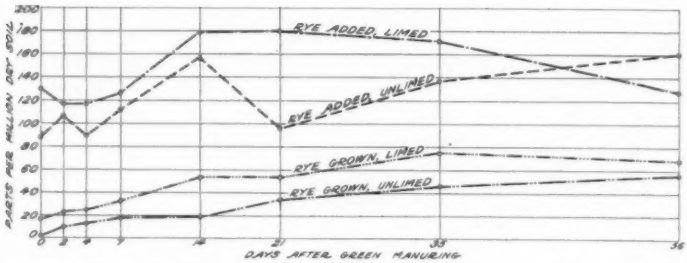

FIGURE 1.—Nitrate nitrogen in the limed and unlimed fallow soil to which rye tops were added and in the limed and unlimed soil on which rye was grown and turned under

shows. Under vetch at the beginning of the experiment there were 25 parts per million of nitrate nitrogen in the unlimed soil and 49 parts per million in the limed soil, in contrast to the 2 and 17 parts per million, respectively, found under rye at the same time.

The turning under of rye tops caused a decrease in the nitrate content of the limed soil at two and four days. (Fig. 1.) On the seventh day the nitrates again began to increase and by the fourteenth day had reached 179 parts per million. They remained at

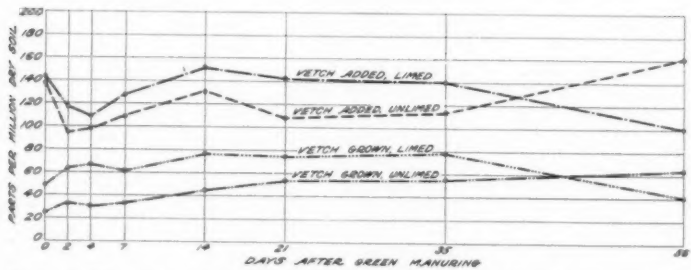

FIGURE 2.—Nitrate nitrogen in the limed and unlimed fallow soil to which vetch tops were added and in the limed and unlimed soil on which vetch was grown and turned under

about this level until the last sampling, when they were the same as at the beginning. On the unlimed soil, the accumulation of nitrates in general showed the same tendencies as in the limed soil until the twenty-first day. At that time, for some unexplained reason, a smaller amount of nitrates was found. At the last two samplings increasing quantities were noted, the maximum of 160 parts per million occurring at the end.

The amounts of nitrogen in the rye tops added to the limed and unlimed fallow soil were 89 and 78 pounds per acre, respectively.

(Table 1.) After 14 days the increase in nitrate nitrogen in these two soils amounted to about 98 and 138 pounds per acre, respectively, assuming that an acre of soil weighs 2,000,000 pounds. It would, therefore, appear that in the limed soil all the nitrogen in the rye tops was nitrified within the 14-day period and that in the unlimed soil some of the soil nitrogen was nitrified in addition to that in the rye tops.

The effect on the nitrate content produced by turning under the rye which had grown on the unlimed and limed soils is also shown in Figure 1. It is interesting to note that the two curves parallel each other to a remarkable degree, the limed soil always containing more nitrate than the unlimed. But the difference in the two curves is not so great as might have been expected from the fact that the limed soil had a pH value of 7.4, whereas that of the unlimed soil was 4.6. (Table 2.) The amount of nitrogen in the green-manure tops, or its equivalent, was nitrified in 21 days in the unlimed soil, and in 14 days in the limed soil. The nitrates produced later must have come from the decomposition of the roots or of the soil organic matter. No appreciable decrease in nitrates in the limed soil occurred at the end of the experiment, as when rye tops were added to limed soil.

TABLE 2.—*pH values of samples from fallow soil to which rye and vetch tops were added and from planted soil where rye and vetch were grown and turned under limed and unlimed*

Days after green manuring	pH values of soils to which rye was added or on which it was grown and turned under				pH values of soils to which vetch was added or on which it was grown and turned under			
	Unlimed		Limed		Unlimed		Limed	
	Added	Grown	Added	Grown	Added	Grown	Added	Grown
0	4.4	4.6	7.5	7.4	4.4	4.4	7.2	7.4
2	4.1	4.6	7.1	7.4	4.2	4.5	7.1	7.1
4	4.1	4.6	6.9	7.4	4.2	4.4	7.1	7.3
7	4.2	4.7	7.0	7.5	4.3	4.5	7.2	7.1
14	4.0	4.6	7.2	7.4	4.1	4.5	7.0	7.2
21	4.1	4.5	7.1	7.4	4.2	4.2	7.2	7.1
35	4.1	4.4	7.0	7.3	4.4	4.3	7.1	7.3
56	4.3	4.3	6.9	7.3	4.2	4.3	7.2	----------

The addition of vetch tops to the unlimed and limed soils caused a more pronounced decrease in nitrates (Fig. 2) during the first few days than was observed in the case of the rye green manuring. The reason for this is not clear. The weight of rye turned under was nearly double that of the vetch in the unlimed soil and more than three times that of the vetch in the limed soil. The percentage of nitrogen in the rye was somewhat lower in both cases (Table 1). These conditions should favor a greater utilization of the soil nitrates by the organisms decomposing the rye than by those decomposing the vetch. At the last sampling the accumulation of nitrates in the unlimed soil and the decrease in the limed soil were rather striking and corroborate the results obtained when rye tops were added to the soils. It should also be noted that none of the nitrogen in the vetch tops added to unlimed soil was nitrified until the last sampling and that only about half the nitrogen added to the limed soil was nitrified, this occurring on the fourteenth day.

The turning under of vetch grown on the limed and unlimed soil (Fig. 2) increased the nitrates, as did rye grown on the soils with the exception of the last sampling of the limed soil. Here again there seems to have been some factor in the limed soil which caused a decrease in nitrates at the end of the experiment, whereas in the unlimed soil this influence was lacking and the nitrates continued to increase. A decrease in soil nitrates immediately after green manuring or some time afterward does not necessarily mean any loss of nitrogen under these conditions. If the nitrogen were not lost, it must, therefore, have been absorbed by soil organisms. Additional work may throw some light upon this point.

EFFECT OF TREATMENTS ON THE PLATE COUNTS OF MICROORGANISMS

The plate counts of the variously treated rye plots are shown in Figure 3. Before green manuring there was no difference in the counts of the limed soils regardless of the fact that rye had been grown on one while the other had remained fallow and that the latter con-

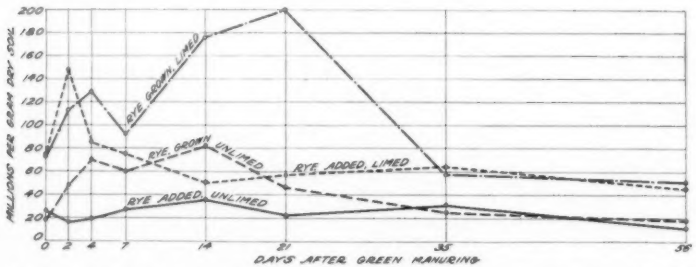

FIGURE 3.—Effect of treatments on the number of microorganisms in the soil as determined by plating on soil-extract agar; rye green-manure series

tained 7.5 times as much nitrate. In the case of the unlimed soil, the plate counts of the cropped and fallow soil were also practically identical, but they were approximately only one-third as large as those of the limed soil.

The addition of rye tops to the limed soil caused a rapid increase in the plate counts within two days (75,000,000 to 148,000,000). At four days a great decrease in the numbers was obtained, 85,000,000 being recorded. The decrease continued more slowly to the fourteenth day, when the counts were only 50,000,000 per gram, or 25,000,000 less than at the beginning of the experiment. Thereafter the counts remained quite stationary. In comparing this curve with those published previously (*8*) it will be noted that the peak of the curve in this experiment occurred at two days instead of at four or seven days. The reason for this may lie in the fact that in this experiment the rye was more succulent and contained only 12.4 per cent dry matter (Table 1), as compared with 25 and 20.7 per cent in the previous experiments. Furthermore, the dry weight of the green manure added to the soil was only about one-third as great as that of the green manure used previously. In view of these facts, it seems reasonable to suppose that the decomposition would have been more rapid under these conditions. However, the reason for the plate counts

falling below those obtained at the beginning of the experiment and remaining so is not clear, and no explanation is offered to account for it.

When a similar amount of green rye was added to the unlimed soil (pH 4.4) there was a reduction in numbers on the second day from 26,000,000 to 16,000,000. This was followed by a very slow increase to a maximum of 35,000,000 at 14 days. These variations are not considered significant since much larger variations are generally obtained. The failure of a green manure to increase the plate counts was noted before (*8*). The explanation for this must await more detailed experiments.

When rye which had grown on the limed soil was turned under, a definite increase in plate counts took place at two and four days. (Figure 3.) From the peak of 129,000,000 at four days the counts dropped to 92,000,000 at seven days. In general, these counts are similar to those previously reported (*8*). However, after the decrease on the seventh day an enormous increase was observed. The counts

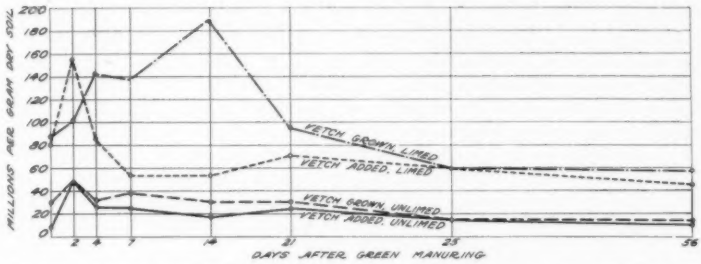

FIGURE 4.—Effect of treatments on the number of microorganisms in the soil determined by plating on soil-extract agar; vetch green-manure series

obtained on the fourteenth and twenty-first days were very unexpected and will be discussed later in connection with other factors.

The rye grown on the unlimed soil when turned under produced an effect on the plate counts similar to that observed on the limed soil with one exception. In this case the peak (82,000,000) came at 14 days instead of at 21 days. The counts dropped to 46,000,000 at 21 days and to approximately 20,000,000 at the last two samplings; that is, the same as at the beginning. As compared to the counts on the corresponding limed plot all the counts on this unlimed soil were considerably lower.

The effect on the plate counts of the addition of the vetch tops to the limed soil is shown in Figure 4. The initial count of 80,000,000 was practically doubled in two days by the addition of vetch tops. On the fourth day, the counts were back to 85,000,000 and on the seventh and fourteenth days they were down to 55,000,000. Thereafter they remained low, the final count being 45,000,000. This curve is practically identical with that curve showing the counts when rye tops were added to the limed soil. (Fig. 3.) The agreement of these results strengthens the belief that the decrease in counts is not accidental, but is the result of some factor outside the scope of this investigation.

The addition of the vetch tops to the unlimed soil (Fig. 4) caused a relatively large increase in the plate counts (from 9,000,000 to

48,000,000) during the first two days. On the fourth day the number had decreased to 26,000,000. Thereafter the general tendency was downward, the last count being the same as the first.

The curve showing the plate counts made on the unlimed soil where vetch was grown and turned under parallels fairly closely the curve just discussed. At the beginning the counts on the planted soil were higher, but this difference was wiped out on the second day. On the seventh and fourteenth days there was some indication that the decomposition of the roots had some influence in keeping the count from decreasing as fast as it did when only the tops were added. During the remainder of the experiment the counts on both unlimed plots were low and practically identical.

The decomposition of the vetch grown upon the limed soil proceeded in general as did the rye, when measured by the plate counts. The chief differences noted were that the highest count obtained on the vetch plot came on the fourteenth day instead of on the twenty-first and that the decrease thereafter was not quite so rapid.

One of the interesting facts brought out in Figures 3 and 4 is the secondary increase in plate counts from the limed soils where rye or vetch had been grown and turned under. Since the green weight of the tops in these cases was the same as when rye and vetch tops were added to the fallow soil, it would appear that the effect on the plate counts was due to the decomposition of the roots. Investigators disagree as to the relative proportion of tops to roots, and with very good reason. The type and fertility of the soil, temperature and moisture, and the nature and age of the plant, all affect this relationship. Average figures (*6, p. 74*) show that the roots of vetch comprise 17 per cent of the total crop. But since the amount of roots is known to vary widely, it can only be estimated that the cropped soil had from one-third to one-tenth more material to be decomposed than did the unplanted soil which received the rye and vetch tops. This amount of material would not account for the greater number of organisms, for the following reasons. The dry weight of and the nitrogen in the rye tops grown on the limed soil were about 77 and 73 per cent, respectively, of the dry weight and nitrogen in the tops added to the limed fallow soil, whereas in the vetch experiment the relative amounts were reversed; i. e., where vetch was grown on the limed soil, the dry weight of the tops was nearly three times and the nitrogen two times that in the tops added to limed fallow soil. (Table 1.) This discrepancy in the dry weights and nitrogen content of the tops grown on and those added to the limed soil was due to a difference in the composition of the green manure (the green weight being the same) and was unavoidable under the circumstances. However, the fact that the curves showing the plate counts in the two experiments (figs. 3 and 4) agree so closely, proves conclusively that the variation in the amounts of dry matter or nitrogen in the green manures had no effect upon the plate counts.

Another interesting fact is brought out in Figures 3 and 4, that is, the reduction in the plate counts which inevitably occurs, provided, of course, that the treatment produced an initial increase in the number of microorganisms. For instance, where rye and vetch tops were added to fallow limed soil, the reduction came at 7 or 14 days, and where the green manures were grown on the limed soil

and turned into it, it occurred in 35 days. Similar results were previously reported (*8*) after the second application of green manure to this soil. However, the first application of green manures, made soon after this soil was brought into the greenhouse, showed a tendency to produce a secondary rise in numbers, which then remained high. The data at hand do not warrant conclusions as to the possible causes of the inhibition of the numbers of microorganisms.

The lack of an increase in plate counts due to the growth of the rye or vetch is quite definite and is in agreement with other work (*10*). In only one of the four cases was there any difference between the counts made on the soil that remained fallow and that which had grown rye or vetch.

EFFECT OF TREATMENTS ON THE FUNGI PRESENT IN THE SOIL

The decomposition of rye green manure grown on the limed and unlimed soils increased the number of fungi to only a slight degree.

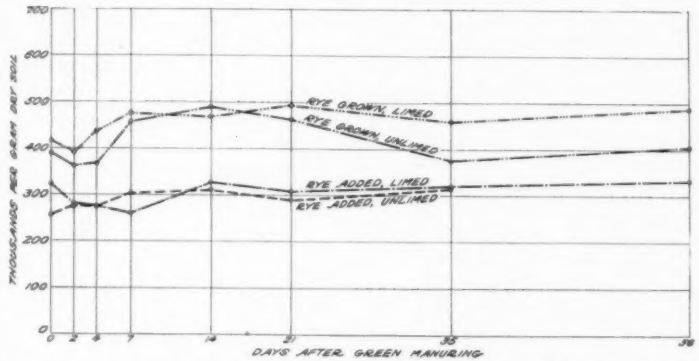

FIGURE 5.—Number of fungi in limed and unlimed fallow soil to which rye tops were added and in limed and unlimed soil on which rye was grown and turned under

(Fig. 5.) At all samplings except one the limed soil contained a few more fungi than the unlimed soil, but since the difference in the counts between the limed and unlimed soil at any sampling was less than 100,000, and since the maximum increase during the experiment was only about 120,000 per gram, it is evident that the fungi can not be considered as important agents in the decomposition of green manures under these conditions.

The decomposition of rye tops added to the limed and unlimed fallow soils produced even less effect upon the number of fungi (Fig. 5) than did the decomposition of the whole plant, as noted above. The only interesting fact brought out in these figures seems to be that a higher count of fungi was obtained on the soil where the rye had grown than on the fallow soil. These results are in accord with those of Starkey (*10*), who also reported slightly higher counts of fungi under growing plants.

The turning under of vetch where it had grown on the limed and unlimed soil also affected the number of fungi only slightly. (Fig. 6.) Here again the limed soil contained the greater number except in one

case. This may perhaps be explained by the fact that a greater development of the vetch took place on the limed soil, and consequently there was a larger quantity of roots in the soil, which apparently increase the number of fungi to a slight extent (*10*). The same explanation would apply to the rye green-manuring experiment discussed above.

The addition of the vetch tops to the limed and unlimed fallow soils also had practically no effect upon the count of fungi. (Fig. 6.) However, if these curves are compared with those showing the counts on the soil where the vetch had grown, it will be noted that the latter are more than twice as great as the former. This difference between the fungi in the fallow and cropped soils is greater in the vetch than in the rye series and seems to be significant.

In general, turning under rye or vetch, whether grown on the soil or whether grown elsewhere and added to fallow soil, produced very

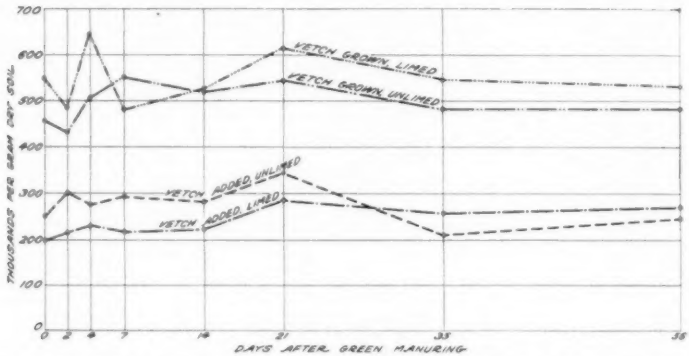

FIGURE 6.—Number of fungi in the limed and unlimed fallow soil to which vetch tops were added and in limed and unlimed soil on which vetch was grown and turned under

little change in the number of fungi. However, the growth of each green manure caused a small but definite increase in the fungi, the increase being more noticeable under vetch. Furthermore, the limed soil, supporting a more vigorous plant growth than the unlimed soil, contained a greater number of fungi. On the other hand, when vetch tops were added to the fallow soil, the greater number of fungi usually occurred in the unlimed soil.

EFFECT OF TREATMENTS ON SOIL ACIDITY

It was noted previously (*8*) that the decomposition of large amounts of green rye or vetch tops showed a tendency to lower the acidity by the production of ammonia during the first week. Afterwards, the reaction became progressively more acid as the ammonia was oxidized to nitrate. The changes in reaction were greater in a fine sandy loam than in the clay loam.

In the present experiments when the same clay loam was used as that employed in the earlier experiments, green manuring produced no definite change in the hydrogen-ion concentration of the soil. (Table 2.) Where rye tops were added to unlimed soil, there

seems to have been an increase in acidity from the second to the thirty-fifth day sampling and less acidity at the last sampling. In the limed soil also rye tops seem to have reduced the pH value from 7.3 to 6.9 in four days. Whether these figures are reliable remains for further proof. For the present let it be said that owing to the high buffer capacity of this soil and the difficulty of obtaining a truly representative sample, no conclusions can be reached as to the effect of decomposing green manure on soil acidity under greenhouse conditions.

One fact does stand out in Table 2, namely, that the growth of rye slightly decreased the acidity of the unlimed soil and slightly increased the alkalinity of the limed soil. This condition persisted throughout the experiment and seems to be very definite. Similar results have recently been reported by Joachim and Panditteseker (*3*). They found a reduction in acidity from one year to the next in soil on which green manures were grown.

The growth of vetch, on the other hand, seems to have had no effect on the pH values of the limed soil and only a slight effect on those of the unlimed soil.

EFFECT OF TREATMENTS ON CARBON DIOXIDE PRODUCTION

In recent years, the production of carbon dioxide in soil has been studied to an increasing extent. Investigators have attempted to correlate it with crop yields, nitrate accumulation, and bacterial numbers, with varying success. There are four general methods of determining carbon dioxide: (1) Sampling the soil air, (2) drawing purified air through the soil and absorbing the carbon dioxide, (3) passing purified air over the soil and absorbing the carbon dioxide, and (4) collecting the carbon dioxide evolved from the soil by placing a respiratory chamber over the soil and sampling the gas. Each method has some advantages and some disadvantages. In this work, a combination of methods 3 and 4 was used, and while the method is not perfect it is inexpensive and fairly reliable and is best suited to the conditions under which this work was done.

The amount of carbon dioxide evolved daily from a square meter of soil in the rye green-manure experiment is shown in Figure 7. The soil supporting the growth of rye was evolving more carbon dioxide before the turning under of the rye than was the corresponding unplanted soil, and the limed soil was giving off considerably more than the unlimed. Similar observations have been made (*9*, *11*) and need not be discussed at this time.

Turning under the rye grown on the limed soil stimulated the production of carbon dioxide during the first day. During the second day there was a slight decrease. This was followed by a considerable increase during the third day, reaching the peak of approximately 19 gm. Thereafter the rate decreased quite steadily to the twelfth day. At this time the amount of carbon dioxide (4 gm.) was slightly less than it had been before the rye was turned under. The amount increased on the eighteenth day and remained above that evolved from the other soils during the rest of the experiment.

Turning under the rye grown on the unlimed soil produced an effect similar to that on the limed soil but to a less degree. In this case the peak of 15 gm. of carbon as carbon dioxide was reached on the fifth

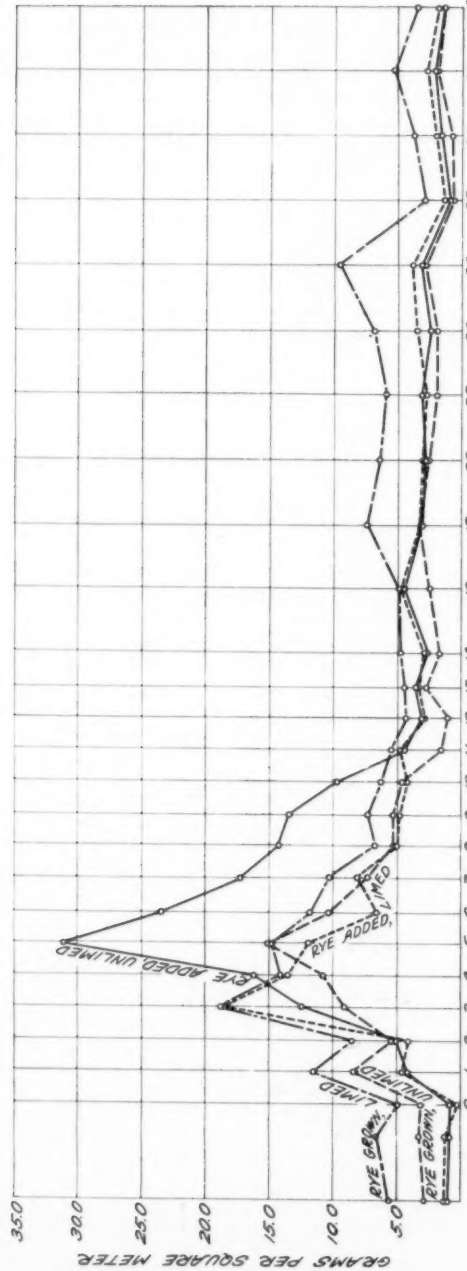

FIGURE 7.—Carbon as carbon dioxide evolved from the limed and unlimed fallow soil to which rye tops were added and from the limed and unlimed soil on which rye was grown and turned under

day, whereas in the former case a peak of 19 gm. was reached on the third day. The decrease in the evolution was very rapid, the low amount of 1 gm. being reached on the twelfth day. Thereafter this amount remained for the most part below the original level.

The evolution of carbon dioxide from the limed soil receiving rye tops jumped from 1.5 to 19 gm. during the first three days after treatment. The decrease following this was abrupt but did not reach the level observed before treatment until the twenty-eighth day.

Rye tops added to the unlimed soil caused the greatest evolution of carbon dioxide of all the treatments. The amounts increased each day to a peak of 31 gm. at five days and then decreased until the twelfth day, when 3 gm. were evolved. The reason for this large amount of gas is not clear. It may have been due to some physical factor, such as a larger amount of decomposing material underneath the collecting box, or a chimney effect under the box which collected carbon dioxide from a greater mass of decomposing material than was represented by the size of the collecting box. However, it will be recalled that the plate counts failed to increase in this case, which might indicate that a biological rather than a physical factor was responsible for the aberrant results.

The carbon dioxide evolved from the decomposing vetch under the four conditions is shown in Figure 8. In all cases it increased during the first day, usually decreased on the second day, and then increased on the third and fourth days. The maximum production per day from the limed soil occurred at three days, whereas that from the unlimed soil occurred at five days. Decreasing amounts were obtained until the twelfth day, when only about 3 gm. per day were evolved. Thereafter the evolution remained more

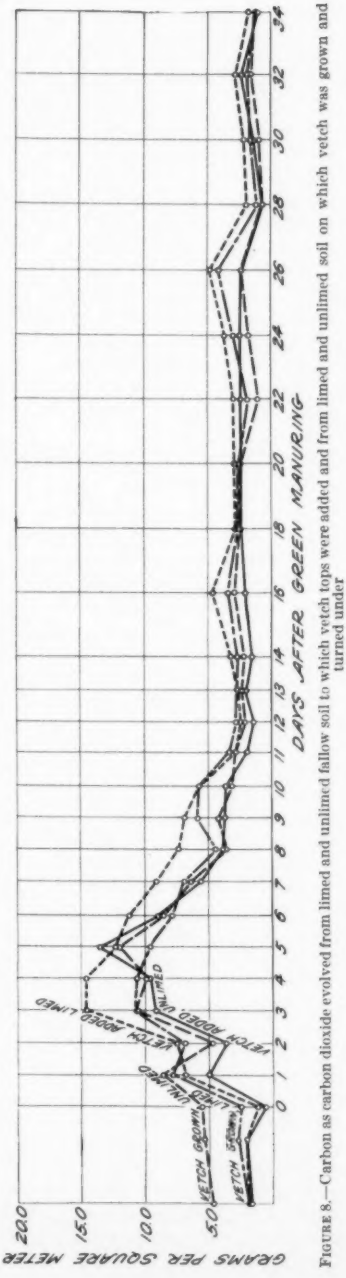
GRAMS PER SQUARE METER
200
150
100
50
DAYS AFTER GREEN MANURING
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 18 20 22 24 26 28 30 32 34
VETCH ROOTS LIMED
VETCH ROOTS UNLIMED
VETCH TOPS LIMED
VETCH TOPS UNLIMED

FIGURE 8.—Carbon as carbon dioxide evolved from limed and unlimed fallow soil to which vetch tops were added and from limed and unlimed soil on which vetch was grown and turned under

or less constant, the limed soil showing a tendency to give off slightly more of the gas. It should be noted that where vetch tops were added to the fallow soil more carbon dioxide was evolved from the limed than from the unlimed soil.

Hill (*1*), using small amounts of soil and various green materials, measured the carbon dioxide evolved daily under laboratory conditions. The largest amounts were given off on the fourth day with both rye and vetch; the amounts then decreased as rapidly as they had increased and became fairly uniform. Vetch gave off much more than rye. The conditions under which Hill's experiments were made were quite different from those reported in this paper. Although the results of the two are similar so far as the peak of production is concerned, they differ in the relative amounts given off from the rye and vetch and in the rate of evolution of the gas. The high nitrate content of the soil used in the writers' experiments may have hastened and intensified the evolution. Other workers (*6*, *12*) have also found a rapid evolution of carbon dioxide in one or two days, usually in closed systems and under laboratory conditions.

NUMBER OF MICROORGANISMS IN RELATION TO NITRATES AND CARBON DIOXIDE

A number of reports (*12*, *p. 717–725*) indicate that a relationship between number of microorganisms, nitrate accumulation, and carbon dioxide production in soil may be expected under certain conditions. It therefore seemed advisable to determine what relationships exist under the conditions of these experiments.

If a comparison is made of Figures 1, 3, and 7, representing, respectively, nitrate accumulation, plate counts, and carbon dioxide production in the limed soil on which rye was grown and turned under, no definite relationship of the three factors is found. However, if the two curves showing the plate counts and carbon dioxide production be compared it will be seen that the first peak of the plate counts at four days is correlated with the peak of carbon dioxide evolution. But the relationship seems to end there, for the second and much higher peak in the numbers curve occurring at the twenty-first day corresponds to a much lower part of the carbon dioxide curve. The nitrate curve, which shows practically a steady increase to the thirty-fifth day, is independent and shows no correlation with the curves representing plate counts or carbon dioxide evolution.

The same relationship between the plate counts, nitrate accumulation, and carbon dioxide production existed in the unlimed soil receiving the same treatment. The initial increase in the plate counts can be correlated with the increase in carbon dioxide. But the peak of the plate-count curve occurring at 14 days corresponds to a lower portion of the carbon dioxide curve. The nitrate curve, as in the preceding experiment, failed to show any relationship to the plate count or carbon dioxide curve.

On the other hand, when rye tops were added to the limed fallow soil there was a good correlation of the three factors. On the second and fourth days nitrates were low, the microorganisms on the second day and the carbon dioxide on the third day were high. On the fourteenth day the nitrates were high, plate counts and carbon dioxide low—as close a correlation as could be desired in such experiments.

When rye tops were added to the unlimed soil very different results were obtained. The plate counts having varied only a little, contrary to the usual behavior, there appeared to be practically no correlation of these with the large variations in the nitrate content and with the carbon dioxide evolution. Since the carbon dioxide evolved in this case was very much greater than in any other experiment and since the number of microorganisms failed to increase, it may be assumed that some factor entered this experiment which was not present in the other. Therefore, a correlation or a lack of correlation is of little importance in this case. It is hoped that additional work along this line may explain these exceptional results.

In the experiments where vetch tops were added as the green manure the same correlations or lack of correlations were noted in case of the limed soil. There was an initial increase in the plate counts and in carbon dioxide evolution and an accompanying decrease in nitrates. After the first few days no further relationship between the curves was noticed. Similar relationships were noted when vetch tops were added to the unlimed soil.

The same relationships were observed when vetch was grown on the limed soil and turned under as when rye was grown. But in the unlimed soil, the effects of the vetch green manure were not the same as the rye, no secondary increase in plate counts occurring. This was due, perhaps, to the weaker growth of the vetch as compared to the rye, which grew well on the unlimed soil, and consequently there was less root material to be decomposed.

Since the writers have no data upon the carbohydrate content of the green manures used or upon the actual rate of its decomposition, the source of the carbon dioxide can not be stated. Waksman and Tenney (*13*) found in laboratory experiments that the soluble materials were decomposed first, and then pentosans and cellulose. More carbon dioxide was produced from young than from old plants during 28 days. Under the conditions of their experiments, the rate of evolution was quite uniform, in contrast to that reported above. However, it would seem logical to assume that the rapid rise in carbon dioxide during the first days of the decomposition of rye and vetch was due mainly to the fermentation of soluble carbohydrate material rather than to the respiration of microorganisms or to the destruction of cellulosic material. It is true that the number of microorganisms increased in seven of the eight cases during the first two days, and while there was some increase in carbon dioxide at that time it was not so great as at four or seven days. At this time the number of microorganisms had decreased to the level or below the level obtaining at the start in four of the eight treatments. It might be said that at the death of the microorganisms carbon dioxide was liberated. That this explanation is inadequate is shown by the following facts. In those cases where the green manure was grown on the limed soil and turned under the great reductions in the number of microorganisms occurred at 21 or 35 days without any accompanying increase in carbon dioxide at that time. Therefore, under these conditions, there seems to be very little correlation between number of microorganisms and carbon dioxide production, nor is there an inverse ratio between number of microorganisms and nitrate accumulation except in the first stages of the decomposition of the green manure added to fallow soil.

CONCLUSIONS

Whether rye or vetch tops are grown elsewhere and added to a fallow soil or whether they are grown on the same soil and turned under makes no difference in the amount or rapidity of the evolution of carbon dioxide. However, the effect on the soil microflora and nitrates is very different under the two conditions. Therefore, if observations are to be made upon the effect of green manuring on the soil microflora or on the accumulation of nitrates, the green-manure crop must be grown on the soil into which it is turned.

SUMMARY

Microbiological studies on the decomposition of green rye and green vetch tops grown elsewhere and turned under 5 inches below the surface in limed and unlimed fallow Leonardstown clay loam high in nitrates gave the following results under greenhouse conditions:

Nitrate nitrogen as a rule decreased during the first 4 days and then increased to a peak at 14 days. In the limed soil the nitrates decreased at the end of the experiment, whereas in the unlimed soil they increased and finally surpassed the amount present in the former.

Plate counts of microorganisms in the limed soil showed an immediate increase followed by a greater decrease. In the unlimed soil rye tops failed to increase the comparatively low numbers, whereas vetch showed a slight initial increase.

The number of fungi and the pH value of the soil were not affected by the green-manure treatment.

The evolution of carbon dioxide was rapid, the greatest amount being given off on the third or fourth day after green manuring. After 12 or 14 days, the amounts given off were only slightly higher than that given off by the untreated soil.

During the early stages of the decomposition of rye and vetch tops in the limed and unlimed soil there was a direct ratio between the number of microorganisms and the carbon dioxide production and an inverse ratio between these and nitrate content. After decomposition was well advanced the same relationships were noted when rye tops were added to the limed soil, but not when they were added to unlimed soil.

Simultaneously, other studies were made on the same type of soil. In these studies the green-manure crop was grown on the soil into which it was turned 5 inches deep. The following results were recorded:

Nitrates were much lower under the growing plants than in the fallow soil; lower under the rye than under the vetch. After the crops were turned under there was a steady increase in nitrates in all cases. The limed soils showed a tendency to have less nitrate at the end than they had at 35 days, whereas the unlimed soils showed a steady increase to the end of the experiment.

The growth of the green manures did not affect the number of microorganisms, but in limed soil the decomposition of the roots and tops produced a secondary increase after the primary increase noted when only tops were added to the soil. Similar conditions prevailed in the unlimed soil of the rye series. But in the unlimed soil of the vetch series no effect of the decomposition of the roots on the plate counts was observed.

The number of fungi was considerably greater in the soil where rye and vetch had been grown than in the fallow soil. However, turning under the green manure failed to increase these numbers. The pH value of the soil was likewise not changed.

The evolution of carbon dioxide proceeded as it did when only tops were used, the unlimed soils producing the maximum amounts one or two days later than the limed soil.

During the first few days of the decomposition, the production of carbon dioxide varied directly with the plate counts; but nitrate accumulation was not inversely proportional as was the case when only tops were added to fallow soil. During the remainder of the decomposition period no relationships were observed.

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METHODS OF ESTIMATING COTTON FIBER MATURITY[1]

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INTRODUCTION

The spinner of cotton yarns finds cotton containing an abnormal quantity of immature fibers to be wasty and unduly "neppy" or full of knots and snarls which can not all be removed. (Fig. 1, A.) The neps removed during the spinning process are accompanied by a high percentage of good fibers that have become entangled in them. (Fig. 1, B.) The labor involved in the spinning of yarn from "neppy" cotton is very materially greater than that required for cotton containing a limited amount of immature fibers. The larger neps often catch in the combs, causing the yarn to break. Yarn from cotton that is comparatively free from neps is smooth running, free from neps, more easily sold, and commands a better price. (Fig. 2.) All cotton contains some immature fibers, and normal amounts do not seem to lower the spinning quality materially according to Clegg.[2]

A sufficient number of spinning tests have not been made with cotton containing a known amount of immature fibers to establish the limits allowable before the character of the yarn is impaired. Present data indicate that from 25 to 30 per cent immaturity is not harmful, dependent largely upon the amount of extremely thin-walled or "dead" fibers present. It is highly important that some simple but reasonably accurate method of determining the presence of abnormal quantities of immature fibers be devised as an aid in determining the factors affecting immaturity.

MICROSCOPICAL EXAMINATION OF FIBERS

The Shirley Institute of Didsbury, Manchester, England[3] suggests the use of an 18 per cent solution of sodium hydroxide in detecting immature fibers. The fibers are mounted on a glass slide and flooded with the sodium hydroxide solution. This caustic solution removes the waxy covering of the fibers and permits the absorption of water by the fiber walls. Microscopical examination shows that the thick-walled mature fibers will absorb sufficient water to cause them to straighten out into rodlike forms while the extremely thin-walled immature or dead fibers will retain their twisted forms. (Fig. 3.) Fibers with intermediate wall thickening will retain some convolutions, the amount of twisting being dependent upon the thickness of the fiber walls.

While this method is somewhat slow and tedious it does have sufficient accuracy to warrant its use when representative samples of cotton are classified. It has been used by the writer in working out other methods for evaluating cotton from the standpoint of maturity of the fibers and in determining some of the factors respon-

[1] Received for publication Apr. 15, 1931; issued November, 1931.

[2] CLEGG, G. G. IMMATURITY OF COTTON. Empire Cotton Growing Corporation. Conference on Cotton Growing Problems. Rpt. and Summary Proc. August, 1930: 13–17. London. 1930.

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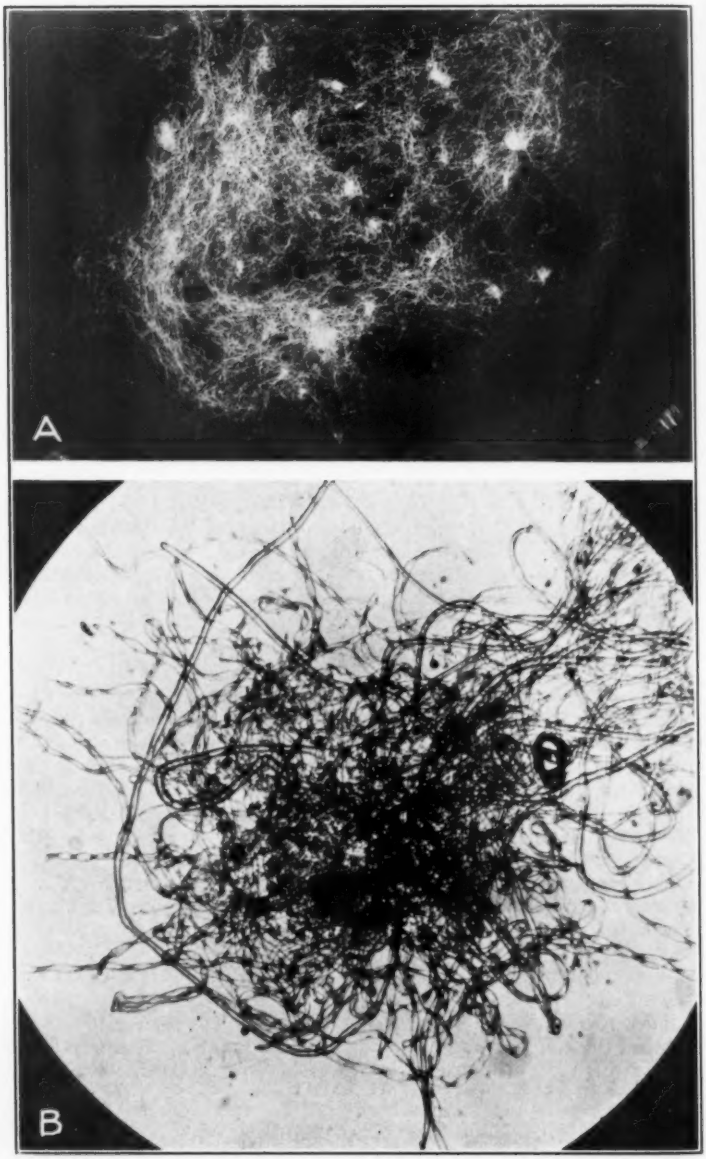

FIGURE 1.—A, Neps or knots in cotton lint, formed largely from immature fibers; B, photomicrograph of a nep treated with caustic soda solution. Immature fibers retain twist while mature fibers become rodlike. (Highly magnified.)

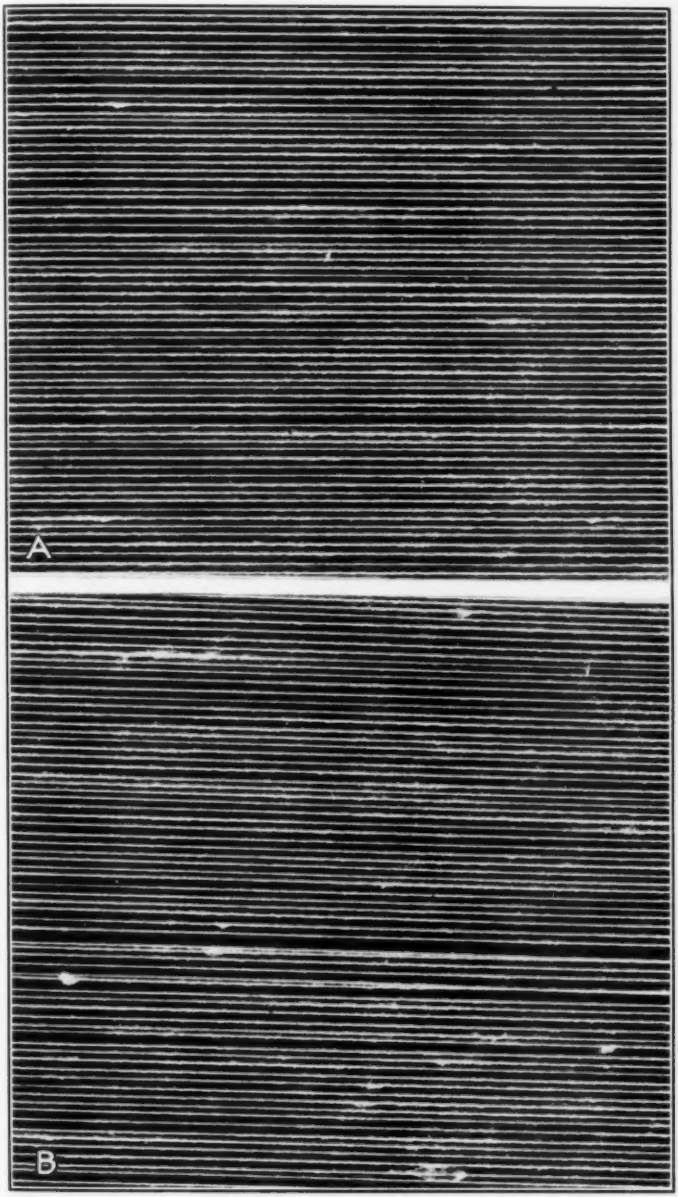

FIGURE 2.—Yarn spun from cotton comparatively free from neps (A), and from cotton containing numerous neps (B)

sible for the presence of abnormal quantities of immature fibers. From 1,500 to 2,000 fibers were treated and classified for each determination. When fibers of individual seeds were classified 500 fibers were used. Exclusive of the varietal tests all determinations were made with Acala cotton.

FIGURE 3.—Photomicrograph of cotton fibers treated with caustic soda solution. Fibers *a* and *c*, intermediate in wall-thickening, are classed as immature; fibers *b* and *d* are thick-walled and mature; fiber *e* is very thin-walled, dead, and immature

FIBER MATURITY IN RELATION TO BOLL FLUFFINESS

A number of research workers including Balls [4] and Hawkins and Serviss [5] have reported data showing that cotton fibers attain practically their full length following fertilization before thickening of the fiber walls begins. Consequently cotton bolls which open prematurely, because of any abnormal interferences, may contain fibers of normal length, and yet wall thickening may have been seriously

[4] BALLS, W. L. THE DEVELOPMENT AND PROPERTIES OF RAW COTTON. p. 73–85, illus. London. 1915.
[5] HAWKINS, R. S., and SERVISS, G. H. DEVELOPMENT OF COTTON FIBERS IN THE PIMA AND ACALA VARIETIES. Jour. Agr. Research 40: 1017–1029, illus. 1930.

impaired. A large percentage of such prematurely opened bolls contain matted locks which do not fluff well in opening. (Fig. 4.)

During the season of 1929, a sufficient number of well-fluffed bolls and also of poorly opened bolls containing matted locks were collected in an experimental field of Acala cotton to supply approximately 150 pounds of lint from each type of boll for spinning tests. A fiber count of a representative sample of fibers treated with caustic soda showed that the fluffy bolls contained an average of 22.9 per cent of immature or thin-walled fibers and the matted bolls 49 per cent of immature fibers. The appeal board of review examiners in the United States Department of Agriculture reported that while the cotton from the fluffy bolls was irregular in length and somewhat

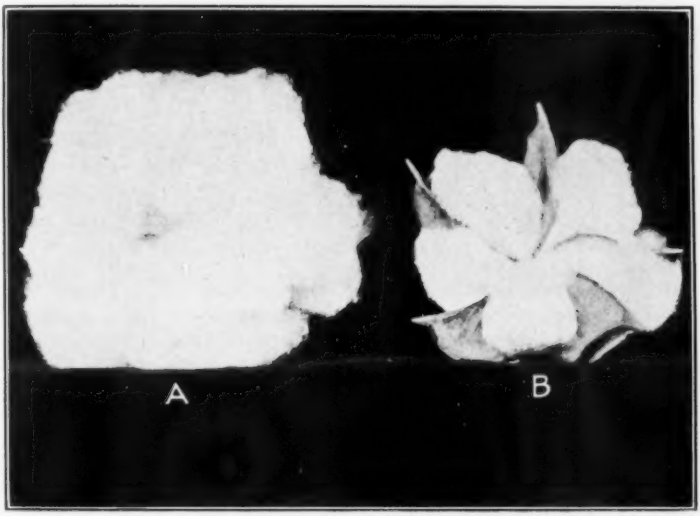

FIGURE 4.—A, Fluffy cotton boll, comparatively free from immature fibers; B, matted cotton boll, high in immature fibers

neppy it had normal strength. (Table 1.) The matted bolls contained cotton that was classified as wasty, exceedingly neppy, and perished as to strength.

TABLE 1.—*Classification of cotton lint by appeal board of review examiners, Washington, D. C.*

Type	Preparation	Grade	Length of staple	Uniformity [a] (length)	Strength [b]	Remarks
			Inch			
Fluffy bolls	Good	3	1	Irregular	Normal	Neppy.
Matted bolls	Poor	5 (spotted)	¾–⅞	Wasty	Perished	Very neppy.

[a] Uniform, regular, irregular, or wasty. [b] Strong, normal, fair, weak, or perished.

EFFECT OF SOIL ALKALI ON FIBER MATURITY

High concentrations of soil alkali have their effect on the maturity of the fibers in the fluffy bolls as well as in the matted bolls. These two classes of bolls were collected from plants growing on soils high

in alkali, soils intermediate in alkali, and from soils very low in alkali and were examined for maturity of fibers. Fiber maturity was definitely correlated with boll fluffiness and soluble-salt content. (Table 2.) All bolls grown on the soils high in alkali contained abnormal quantities of immature fibers irrespective of fluffiness, although the matted bolls contained a much higher percentage of immature fibers than did the fluffy bolls. These soils were so highly impregnated with soluble salts that the plants were greatly stunted, and it was noted that very few bolls opened as well as those on the nonalkali soils.

TABLE 2.—*Percentage of immature fibers produced by cotton grown upon soils containing various quantities of alkali*

Alkali in soil	Fluffy bolls	Matted bolls
High	41.4	56.3
Intermediate	26.0	38.6
Very low	20.6	30.8

FIBER MATURITY IN RELATION TO SEED MATURITY

While fluffiness of the seed cotton still unharvested in the field can be utilized as an index of fiber maturity, it is difficult to ascertain the extent of fluffiness in cotton that has been removed from the boll. The degree of seed development, as indicated by color, size, and plumpness, is correlated with fiber maturity to a large extent. The seed must have their fuzzy coverings removed before these characters can be readily observed. Delinting can be accomplished easily by stirring the seed in concentrated sulphuric acid for a few minutes, followed by a thorough washing in water.

Two distinctly different lots of delinted seed are shown in Figure 5. One lot (fig. 5, A) contains but 0.4 per cent of light-colored, poorly developed seed with 30.7 per cent immature fibers, whereas the other (fig. 5, B) contains 25 per cent of light-colored poorly developed seed with 45.2 per cent immature fibers. In these determinations, in common with all others made by the writer, it has been found that even the darkest-colored, best-developed seeds invariably produce some immature fibers. It has been observed, however, that the best seeds in a lot containing a comparatively high percentage of light-colored and poorly developed seeds, produce a higher percentage of immature fibers, on an average, than do the best seeds in a lot containing a low percentage of light-colored and poorly developed seeds. Table 3 and Figure 6 illustrate this conclusion. Wide variations in fiber maturity occur in seeds of similar appearance. For example, Figure 6, A, shows seeds very similar in appearance with fiber immaturity ranging from 11.8 to 40.4 per cent. Twenty-five dark-colored, well-developed seeds and a like number of light-colored and poorly developed seeds were selected from each of the three pickings from the same plot of cotton. Five hundred representative fibers from each individual seed were classified by the caustic soda method. Table 3 shows that while none of these lots contains so high a percentage of immature seeds as the lot in Figure 5, B, distinct differences in fiber maturity do prevail.

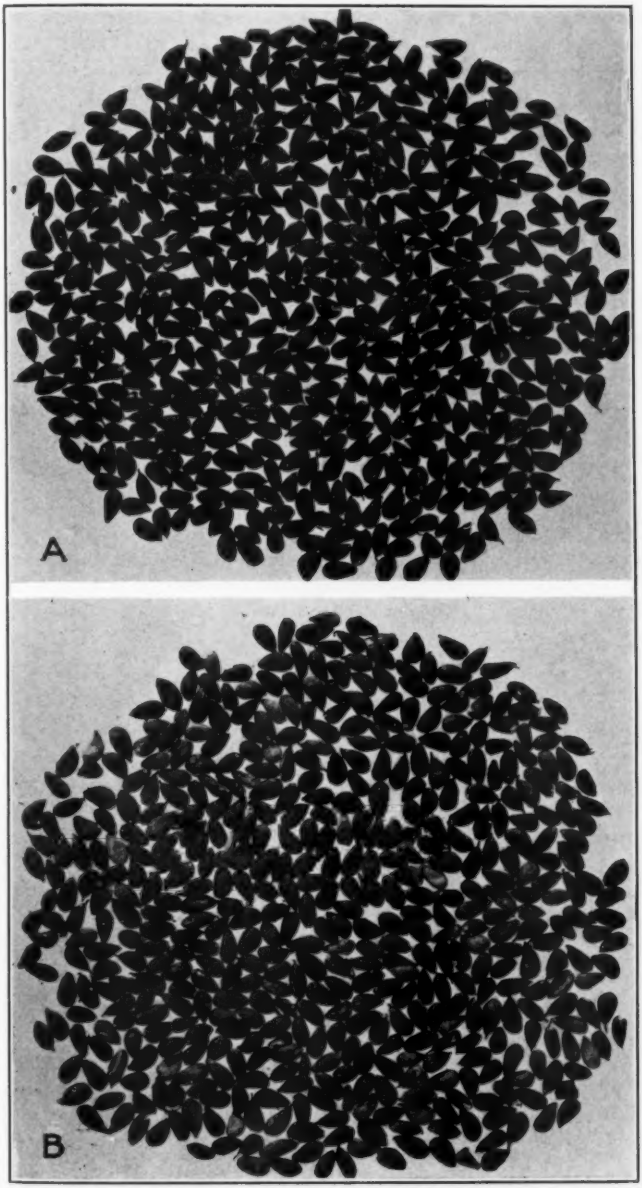

FIGURE 5.—A, Delinted cotton seed, 99.6 per cent dark-colored, plump, and well matured; these seeds produced 30.68 per cent immature fibers. B, Delinted cotton seed, 25 per cent light-colored, immature; these seeds produced 45.18 per cent immature fibers

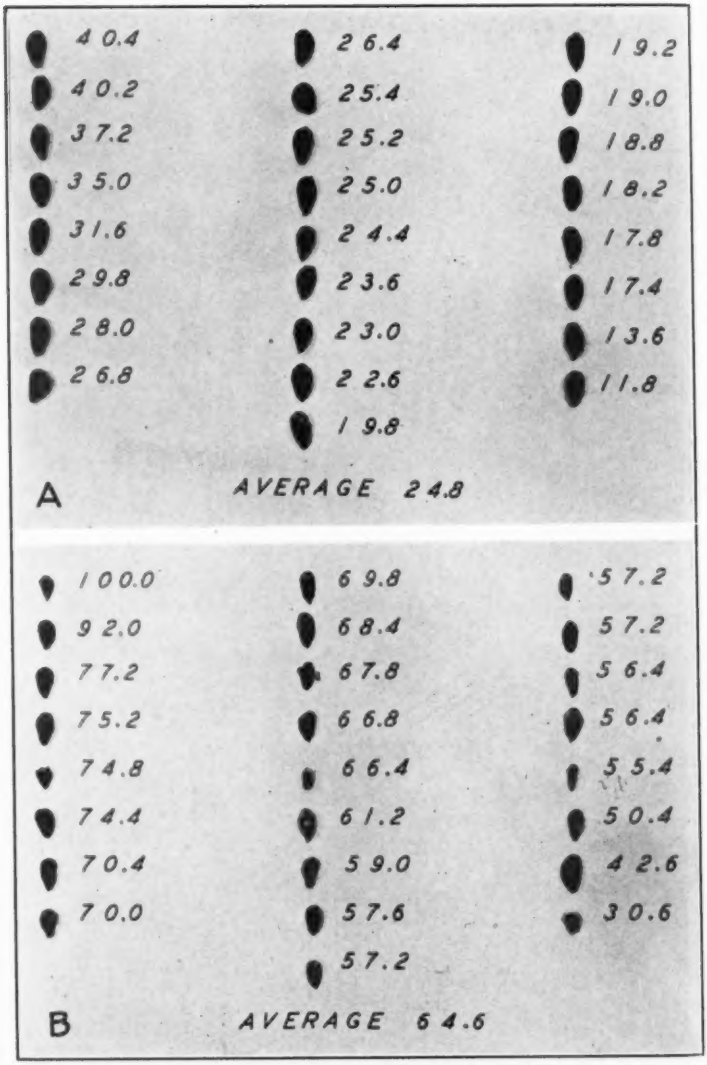

FIGURE 6.—A, Dark-colored mature cotton seed; B, light-colored immature seed. Figures refer to percentage of immature fibers on each seed

TABLE 3.—*Relation between maturity of cottonseed and maturity of fibers*

Picking No.	Dark, well-developed seed	Light poorly developed seed	Immature fibers from—	
			Dark, well-developed seed	Light, poorly developed seed
	Per cent	*Per cent*	*Per cent*	*Per cent*
1	98.98	1.02	24.8	64.6
2	98.80	1.20	25.4	74.9
3	97.42	2.58	26.5	84.0

EFFECT OF TIME OF PICKING ON SEED AND FIBER MATURITY

The third and last picking of cotton usually contains the greatest amount of immature seed as well as immature fibers. Such was the case in the above-mentioned lots and also in most of the numerous other samples examined, data on the latter being given in Tables 4 and 5. The lack of maturity in both seeds and fibers in the last picking is due in part to insect and disease damage, which is usually more pronounced during the latter part of the season, and in part to unfavorable climatic and soil-moisture conditions, all of which interfere with the normal development of the seeds and fibers. Cotton crops planted late and late-maturing varieties are very apt to have a large number of unopened bolls when the plants are killed by fall frosts. Many of these bolls will open later and will contain a high percentage of immature fibers.

TABLE 4.—*Effect of time of picking cotton on seed and fiber maturity*

Picking No.	Light-colored, poorly developed seed	Immature fibers
	Per cent	*Per cent*
1	0.87±0.31	34.30±3.43
2	2.09±1.12	34.88±3.28
3	3.56±1.51	42.31±2.80

FIBER MATURITY IN RELATION TO VARIETAL DIFFERENCES

The Coachella strain of Acala cotton is later in maturing than the other varieties included in Table 5 and had a heavy crop of unopened bolls when the first killing frost occurred, which undoubtedly accounted for the relatively high percentage of immature fibers in the last picking. No explanation can be offered for the high percentage of immature fibers in the first picking of Delfos as compared with the last. It was noted, however, that many of the bolls in the first picking of Delfos opened poorly, and that the lint was exceptionally weak. Evidently distinct varietal differences in fiber maturity are common when varieties are subjected to the same environment.

FIBER MATURITY IN RELATION TO SOIL MOISTURE

The quantity of available soil moisture as well as the time of season during which the bolls are maturing influences boll fluffiness and seed and fiber maturity. Irrigations were applied in this experiment at four intervals, or frequencies, after flowering began, sufficient water being used to moisten the soil throughout the root zone at each irrigation. All plots were irrigated alike prior to the beginning of

flowering. Flowers on 25 representative plants on each plot were tagged daily throughout the season. Fiber analyses were made from lint collected from all bolls maturing on the 25 tagged plants, the flowers of which opened during the weeks ended on the dates specified in Table 6. The data show that the percentage of fiber immaturity became greater as the length of time between irrigations increased. Also the percentage of fiber immaturity increased as the season advanced, irrespective of the frequency of irrigation as shown by the means given for July, August, and September.

TABLE 5.—*Varietal differences in the percentage of immature fibers found in the first and last pickings of cotton*

Variety	First picking (Sept. 29)	Last picking (Oct. 24)
Delfos	46.1	37.5
Oklahoma Triumph	25.1	44.1
Acala (Allen farm)	24.9	40.2
Mebane (Bryant)	21.6	31.6
Acala (Coachella)	25.5	59.4
Average	28.6	43.0

TABLE 6.—*Influence of the frequency of irrigation and time of season on the percentage of immature fibers in cotton*

Weed ended—	Percentage of fiber immaturity under indicated frequency of irrigation			
	10 days	2 weeks	3 weeks	4 weeks
July 7	22.3±0.87	24.5±0.65	25.1±0.82	34.8±1.00
July 14	22.7± .71	20.2± .50	27.9± .69	30.4± .92
July 21	21.6± .90	25.2± .96	28.8± .60	21.5± .49
July 28	23.4± .51	29.0± .87	30.7± .78	28.4± .48
Average	22.5	24.7	28.1	28.8
August 4	23.5± .48	30.1± .88	37.9±1.05	35.8± .48
August 11	23.6± .70	26.5± .82	29.0± .61	26.9± .64
August 18	24.7± .87	27.4± .61	31.4± .68	22.0± .54
August 25	20.0± .98	23.4± .51	23.7± .73	----
Average	23.0	26.9	30.5	28.2
September 1	28.4± .77	31.7± .65	36.7± .84	43.3± .79
September 8	27.8± .70	29.5± .77	29.6±1.29	----
September 15	30.0± .87	33.3± .82	27.7±1.16	41.8± .89
Average	28.7	31.5	31.3	42.6
Average for season	24.4	27.3	29.9	31.7

SUMMARY

The extent of fluffiness in the cotton boll is indicative of the amount of immature fibers present and can be utilized to a certain extent in evaluating cotton from this standpoint while it still remains unharvested in the fields.

The color, plumpness, and maturity of a representative quantity of the delinted seed is also indicative of the percentage of immature fibers borne by the seed, dark-colored, plump, well-developed seed being correlated with a high percentage of mature fibers, and conversely, light-colored, poorly developed seed is associated with a high percentage of immature fibers.

High concentrations of soil alkali, a comparatively low supply of soil moisture, and early frosts are contributing factors in the production of high percentages of immature fibers.

The last picking usually contains the greatest amount of immature fibers.

Distinct varietal differences in fiber maturity are common

THE NUTRITIVE VALUE FOR GROWING SWINE OF THE PROTEINS OF LINSEED MEAL AND OF COTTONSEED MEAL, BOTH ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN [1]

By H. H. MITCHELL, *Chief in Animal Nutrition*, and T. S. HAMILTON, *Associate in Animal Nutrition, Department of Animal Husbandry, Illinois Agricultural Experiment Station*

INTRODUCTION

In a recent contribution from this station, Braman *(2)* [2] described the determination of the digestibility and the biological value of the proteins of cottonseed meal and linseed meal for growing rats. The experiments to be described in this paper represent an extension of this work to growing pigs, for the purpose of detecting any specific differences in the utilization of dietary protein. The existence of supplementary relations between the proteins of these meals and the proteins of corn, when combined in rations in equal proportions, was also studied.

METHODS

Five young Hampshire barrows were used in this experiment. They were confined in metabolism crates very similar in construction to one described by Forbes (*3*). Collections of feces and urine were made daily, and the length of the collection periods was 10 days in all cases, except the last period when the pigs were on a nitrogen-free diet. This was only 7 days in length. The transition periods between collection periods were from 1 to 3 weeks long, and were utilized in adjusting the food intake of the pigs on the new ration to the maximum amount that would be consumed daily. Each pig was kept on the same amount of ration for at least 4 days before the collection of excreta was started.

Aliquots of the urine and washings from the crates were compounded daily for analysis. The feces were collected daily, dried at temperatures of 65° C. or less, ground finely, sampled, and analyzed. Nitrogen determinations were made by a modification of the Kjeldahl method, involving digestion of the sample with sulphuric acid, potassium sulphate, and mercury, liberation of the ammonia produced with a strong alkali-sulphide solution, distillation into 4 per cent boric acid solution, and titration with standard acid, bromphenol blue being used as an indicator.

The experimental rations consisted of a nitrogen-free ration, and three rations of similar character except that some of the starch was replaced by enough of the three feeds tested, i. e., cottonseed meal, linseed meal, and corn, to furnish approximately 8 or 9 per cent of protein. The fat content of the rations, except the nitrogen-free ration, was adjusted to 10 per cent by including various amounts of corn oil, depending upon the determined fat content of the feed contained in them. Two additional rations were prepared by mixing

[1] Received for publication May 26, 1931, issued November, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 748.

in equal amounts the linseed-meal ration and the corn ration in one case, and the cottonseed-meal ration and the corn ration in the other. The exact composition of these rations is given in Table 1. The percentage composition and heats of combustion of the feeds used are shown in Table 2.

TABLE 1.—*Percentage composition and energy content of the experimental rations*

Constituent	Percentage composition of ration No. —					
	1	2	3	4	5	6
Linseed meal		22.90			11.45	
Cottonseed meal			19.37			9.68
Corn [a]				88.50	44.25	44.25
Starch [b]	94	63.17	67.00		31.58	33.50
Minerals [c]	4	4	4	4	4	4
Dried yeast	1	1	1	1	1	1
Cod-liver oil	1	1	1	1	1	1
Corn oil		7.93	7.63	5.50	6.72	6.57
Total	100	100	100	100	100	100
Protein content (N x 6.25) [d]		9.14	9.10	8.66	9.41	8.48
Energy content in calories per gram [d]		4.11	4.09	4.43	4.21	4.16

[a] The corn was dried at a low temperature to increase its protein content.
[b] A high-grade corn starch.
[c] The mineral mixture contained 15 parts each of steamed bone meal, ground limestone (nondolomitic), and sodium chloride, 1.5 parts each of magnesium carbonate and potassium carbonate, 1 part of potassium sulphate, 0.75 part of ferric chloride, and 0.25 part of potassium iodide.
[d] These percentages of nitrogen and gross energy values are the averages of analyses and heats of combustion determined on three mixes of rations 2, 3, and 4. For rations 5 and 6 only one mixture of each was made up and analyzed.

TABLE 2.—*Percentage composition and heats of combustion of the feeds used*

Feed	Percentage of—						Gross energy in calories per gram
	Dry substance	Crude protein	Ether extract	Ash	Crude fiber	Nitrogen-free extract	
Linseed meal	91.30	34.94	4.68	5.25	9.79	36.64	4.36
Cottonseed meal	92.19	41.31	7.09	6.96	9.36	27.47	4.54
Corn, yellow	86.19	8.62	4.06	1.33	3.07	69.11	3.85

The standardizing periods, during which the nitrogen-free ration was fed, were the initial and final periods of the experiment. The experimental collection periods were not started until the nitrogen output in the urine of each pig had reached what appeared to be a constant level as shown by analyses of the day's urine for a number of consecutive days. Such a level was reached after 15 days of feeding in the case of the first standardizing period and after 10 days in the case of the second. The purpose of these periods was to determine the losses of nitrogen in both feces and urine from the bodies of the pigs, in order to estimate, in the periods of nitrogen feeding, the amounts of food nitrogen appearing in feces and urine and measuring the wastage of food nitrogen in digestion and metabolism. The body's contribution to the feces was computed in grams of nitrogen per kilogram of food eaten, and its contribution to the urine was computed in grams of nitrogen per kilogram of body weight.

The experimental rations, including the meals either alone or in combination with corn, were fed in the same periods, one to three of the pigs and the other comparable ration to the remaining two pigs. This plan was followed in order to eliminate from the comparisons as far as possible whatever time changes occurred in the physiological functioning of the pigs with reference to protein (nitrogen) utilization.

RESULTS

The results of the metabolism periods, with the significant intermediate computations, and the final biological values are given in Table 3.

TABLE 3.—*Nitrogen metabolism data and the calculation of biological values of proteins*

Animal No.	Initial weight	Final weight	Food intake	Nitrogen intake	Fecal nitrogen	Metabolic nitrogen in feces	Food nitrogen in feces	Absorbed nitrogen	Urinary nitrogen	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen retained	Biological value of protein
	Kilogram	*Kilogram*	*Kilogram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Per cent*
PERIOD 1, NITROGEN-FREE RATION													
14-30	30.2	31.5	0.91	.....	0.990	[a] 1.09	.....	.....	2.19	[b] 0.0712	.....	.....	.....
23-39	21.1	22.2	.61	.....	.579	[a] .95	.....	.....	1.38	[b] .0636	.....	.....	.....
24-9	28.4	28.8	.66	.....	.812	[a] 1.23	.....	.....	1.86	[b] .0649	.....	.....	.....
14-90	23.8	25.4	.68	.....	.873	[a] 1.28	.....	.....	1.68	[b] .0682	.....	.....	.....
26-39	29.3	31.1	.88	.....	1.107	[a] 1.26	.....	.....	1.88	[b] .0624	.....	.....	.....
PERIOD 2, LINSEED-MEAL RATION													
14-30	35.8	38.1	1.14	16.74	2.42	1.17	1.25	15.49	9.00	2.35	6.65	8.84	57
23-39	24.7	27.2	.88	13.02	1.84	.88	.96	12.06	6.08	1.48	4.60	7.46	62
24-9	33.3	36.3	1.14	16.74	2.27	1.38	.89	15.85	8.64	2.02	6.62	9.23	58
PERIOD 2, COTTONSEED-MEAL RATION													
14-90	29.3	31.8	1.09	15.72	4.18	1.35	2.83	12.89	7.28	1.84	5.44	7.45	58
26-39	36.5	36.7	.91	13.10	3.66	1.12	2.54	10.56	6.01	2.03	3.98	6.58	62
PERIOD 3, COTTONSEED-MEAL RATION													
14-30	41.3	44.2	1.14	16.94	3.43	1.09	2.34	14.60	7.46	2.39	5.07	9.53	65
23-39	29.0	31.1	.68	10.15	2.38	.71	1.67	8.48	4.52	1.52	3.00	5.48	65
24-9	39.5	43.1	1.14	16.94	3.88	1.37	2.51	14.43	7.50	2.11	5.39	9.04	63
PERIOD 3, LINSEED-MEAL RATION													
14-90	33.3	35.4	0.78	10.94	1.51	0.94	0.57	10.37	5.28	1.81	3.47	6.90	66
26-39	38.1	40.6	.91	13.15	1.86	1.09	.77	12.38	6.70	1.91	4.79	7.59	61
PERIOD 4, CORN RATION													
14-30	50.8	54.2	1.36	18.93	3.52	1.22	2.30	16.63	10.34	2.53	7.81	8.82	53
23-39	34.7	35.2	.61	8.36	1.30	.66	.64	7.72	4.42	1.55	2.87	4.85	63
24-9	47.2	48.1	.68	9.65	1.39	.80	.59	9.06	5.82	2.11	3.71	5.35	59
14-90	39.2	39.9	.67	9.24	1.58	.78	.80	8.44	4.93	1.78	3.15	5.29	63
26-39	43.5	45.4	.79	11.03	1.94	.92	1.02	10.01	5.26	1.85	3.41	6.60	66

TABLE 3.—*Nitrogen metabolism data and the calculation of biological values of proteins*—Continued

PERIOD 5, LINSEED-MEAL AND CORN RATION

Animal No.	Initial weight	Final weight	Food intake	Nitrogen intake	Fecal nitrogen	Metabolic nitrogen in feces	Food nitrogen in feces	Absorbed nitrogen	Urinary nitrogen	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen retained	Biological value of protein
	Kilogram	*Kilogram*	*Kilogram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*	*Per cent*
14-90	41.7	42.0	0.60	8.77	1.03	0.67	0.36	8.41	4.16	1.55	2.61	5.80	69
26-39	47.2	48.8	.79	11.95	2.04	.89	1.15	10.80	5.91	1.67	4.24	6.56	61

PERIOD 5, COTTONSEED-MEAL AND CORN RATION

14-30	57.8	61.7	1.36	18.48	3.82	1.14	2.68	15.80	8.55	2.42	6.13	9.67	61
23-39	37.0	38.6	.68	9.22	1.81	.78	1.03	8.19	4.46	1.43	3.03	5.16	63
24-9	49.2	50.6	.79	10.77	2.27	.92	1.35	9.42	5.29	1.87	3.42	6.00	64

PERIOD 6, COTTONSEED-MEAL AND CORN RATION

14-90	43.3	42.4	0.46	5.83	1.45	0.50	0.95	4.88	2.96	1.26	1.70	3.18	65
26-39	51.7	54.4	.91	12.32	2.84	1.00	1.84	10.48	6.45	1.47	4.98	5.50	53

PERIOD 6, LINSEED-MEAL AND CORN RATION

14-30	65.1	69.2	1.36	20.50	3.23	1.06	2.17	18.32	10.23	2.21	8.02	10.30	56
23-39	40.6	42.2	.68	10.23	1.72	.81	.91	9.32	4.90	1.30	3.60	5.72	61
24-9	53.1	55.6	.91	13.66	2.00	1.05	.95	12.71	6.94	1.67	5.27	7.44	58

PERIOD 7, NITROGEN-FREE RATION

14-30	71.2	72.1	1.36	.....	0.959	[a] 0.71	.....	.....	1.82	[b] 0.0254	.....	.....	.....
23-39	42.4	42.0	.51	.....	.631	[a] 1.24	.....	.....	1.05	[b] .0250	.....	.....	.....
24-9	56.0	56.0	.77	.....	.864	[a] 1.13	.....	.....	1.34	[b] .0240	.....	.....	.....
14-90	43.1	43.5	.45	.....	.471	[a] 1.04	.....	.....	.94	[b] .0218	.....	.....	.....
26-39	54.7	53.1	.66	.....	.704	[a] 1.07	.....	.....	1.13	[b] .0210	.....	.....	.....

[a] Fecal nitrogen per kilogram of dry feed. These values were used in estimating the metabolic nitrogen in the feces in periods 2, 3, 4, 5, and 6. The change in the ratio of metabolic nitrogen to dry matter consumed from period 1 to period 7 was assumed to occur in a linear fashion.

[b] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous (body) nitrogen in the urine in periods 2, 3, 4, 5, and 6, the same assumption of a linear variation from period 1 to period 7 being made as in the case of the metabolic nitrogen in the feces.

The biological values calculated for the different rations presumably represent the percentages of the absorbed nitrogen used by growing pigs for both maintenance and growth. These calculations involve the assumption that the excretion of fecal nitrogen per kilogram of food consumed on the nitrogen-free ration in the first and final periods measures the excretion of body nitrogen in the feces in the intervening periods. The change in the excretion of the fecal nitrogen per kilogram of nitrogen-free ration from the first to the seventh period is assumed to occur in a linear fashion with respect to time. The second assumption involved in these calculations is that the excretion of nitrogen in the urine per kilogram of body weight in the first and final periods of nitrogen-free feeding is a measure of the excretion of body nitrogen in the urine in the intervening periods, the change in these values from the first to the seventh period being also assumed to be linear.

For convenience of study, the biological values obtained from the nitrogen metabolism experiments have been collected in Table 4, together with coefficients of apparent and of true digestibility of the dietary nitrogen, allowance being made in the latter case for the metabolic fecal nitrogen.

TABLE 4.—*The digestibility and biological value of linseed and cottonseed proteins when fed to pigs alone and in combination with corn*

	Percentage digestibility and biological value when fed to pig No. —					
	1	2	3	4	5	Average
Linseed protein:						
Apparent digestibility	85	86	86	86	86	86
True digestibility	93	93	95	95	94	94
Biological value	57	62	58	66	61	61
Cottonseed protein:						
Apparent digestibility	80	76	77	73	72	76
True digestibility	86	83	85	82	81	83
Biological value	65	65	63	58	62	63
Linseed protein+corn protein:						
Apparent digestibility	84	83	85	88	83	85
True digestibility	89	91	93	96	90	92
Biological value	56	61	58	69	61	61
Cottonseed protein+corn protein:						
Apparent digestibility	79	80	79	75	77	78
True digestibility	85	89	87	84	85	86
Biological value	61	63	64	65	52	61
Corn protein:						
Apparent digestibility	81	84	86	83	82	83
True digestibility	88	92	94	91	91	91
Biological value	53	63	59	63	66	61

The protein of linseed meal was 94 per cent digestible when allowance is made for the metabolic nitrogen of the feces, while the protein of cottonseed meal was only 83 per cent digestible. The protein of corn was 91 per cent digestible. The greater digestibility of linseed-meal protein than of cottonseed-meal protein is noteworthy and confirms the results of Braman on rats. The mixture of linseed meal and corn also gave a higher coefficient than the mixture of cotton-seed meal and corn, i. e., 92 as compared with 86.

The biological values of all rations, however, were practically the same, i. e., 61 in four cases and 63 for cottonseed meal alone. Working with rats, Braman obtained a much higher biological value of these two oil meals, i. e., 78, though his experiments also indicated practically identical values for the two meals. Bethke and his associates (*1*), also working with rats, have reported average values of 71 to 72, when these oil-meal proteins were fed at a higher level, i. e., 10 per cent. For cottonseed-meal proteins, Nevens (*7*) has reported an average biological value of 66 for three rats, at a level of 10 per cent. It appears probable, therefore, that the pig does not utilize these proteins as well as the rat, although both species utilize the proteins of the two oil meals to the same extent in metabolism.

With respect to corn proteins, however, the biological value of 61 obtained for pigs in this experiment is practically the same as the value previously reported for rats, i. e., 60 (*5*). In other experiments with pigs, Mitchell and Kick (*6*) obtained an average biological value for the proteins of corn of 54.

The results of this experiment do not indicate any supplementary relation between the proteins of cottonseed meal or of linseed meal and the proteins of corn. This is in agreement with the experimental work on rats of Maynard, Fronda, and Chen (*4*) and of Nevens (*7*).

SUMMARY AND CONCLUSIONS

In metabolism studies on five growing Hampshire pigs the digestibility and biological value of the protein (nitrogen) of cottonseed meal, linseed meal and of corn was determined, as well as of mixtures of equal parts of the oil-meal proteins and of corn proteins.

The true digestibility of cottonseed proteins was found to be 83 per cent, that of linseed proteins 94, and that of corn proteins 91. The combinations of cottonseed and corn proteins and of linseed and corn proteins gave average coefficients of 86 and 92, respectively.

The biological values of the proteins of cottonseed, linseed, and corn were practically the same, i. e., 63, 61, and 61. No supplementary relations were established between the proteins of either of these meals and the proteins of corn.

The pig appears to utilize the proteins of corn in metabolism with the same efficiency as the rat, but the pig seems to utilize the proteins of the cottonseed and the flaxseed appreciably less efficiently. For both species, however, the proteins of these two oil-bearing seeds appear to possess equal biological values.

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THE PERCENTAGE OF FAT AS A BASIS FOR ESTIMATING THE COMPOSITION OF MILK[1]

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INTRODUCTION

The fact that the fat of milk is its most easily determinable constituent and that the fat content of milk is closely correlated with its other components, has led to numerous studies having for their object the derivation of formulas expressing these relationships—especially as between the fat and the total solids, the solids not fat, the protein, and the energy.

Most of the published formulas for computing the energy content of milk are based on studies of several breeds together, and have been derived by the employment of energy values computed from the composition of the milk. The present study relates to the milk of Holstein-Friesian cows alone. It covers an entire period of lactation for 12 individuals; the energy values employed are direct bomb determinations; and formulas are also derived for computing the total solids, the solids not fat, and the protein.

EXPERIMENTAL METHODS AND DATA

The analyses on which this paper is based represent 134 samples of milk, each sample being a 28-day composite of aliquots of each milking. The samples were preserved with formaldehyde, as recommended by Palmer (*15*),[2] and were kept in glass-stoppered bottles at a temperature just above freezing.

Total energy was determined by means of an Atwater-Berthelot bomb, with adiabatic features as devised by Fries (*3*). About 5 gm. of milk were weighed into tared platinum ignition crucibles and dried to constant weight, over sulphuric acid, in a vacuum desiccator. The dried milk was then ignited in the bomb calorimeter under a pressure of from 20 to 25 atmospheres of oxygen.

The percentage of fat was determined by the Rose-Gottlieb method on 5-gm. samples.

Total nitrogen was determined by the boric-acid modification of the Kjeldahl-Gunning-Arnold method, with methyl red as an indicator. This procedure is described by Scales and Harrison (*17*).

The statistical treatment of the data was based on the methods discussed by Harris and Benedict (*8*), and by Pearson (*16*).

The analytical data, arranged in the order of increasing fat percentages, are given in Table 1. The results in each case are the average of closely corresponding duplicate determinations. Table 2 summarizes these data by giving the mean content of each component corresponding to intervals of 0.2 per cent of mean fat con-

[1] Received for publication May 8, 1931; issued November, 1931. This paper is based on results incidental to an investigation, as yet unpublished, on the mineral requirements of milk production, conducted under the direction of E. B. Forbes, Director of the Institute of Animal Nutrition of the Pennsylvania State College.

[2] Reference is made by number (italic) to Literature Cited, p. 755.

tent. Table 3 gives the means and standard deviations with their corresponding probable errors and the coefficients of correlation between the percentage of fat and the percentage of other components and energy.

TABLE 1.—*Analyses of 134 samples of milk from 12 Holstein-Friesian cows representing 28-day composites over the entire lactation period*

Fat	Nitrogen	Protein	Total solids	Solids not fat	Energy [a]	Fat	Nitrogen	Protein	Total solids	Solids not fat	Energy [a]
Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories per gram*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories per gram*
2.66	0.417	2.66	10.46	7.80	577	3.33	.476	3.04	12.06	8.73	658
2.69	.456	2.91	11.67	8.99	623	3.34	.445	2.84	12.18	8.84	668
2.70	.424	2.71	10.73	8.03	577	3.35	.487	3.11	12.56	9.21	697
2.70	.405	2.58	10.63	7.93	569	3.35	.451	2.88	12.19	8.84	674
2.71	.439	2.80	10.86	8.15	584	3.36	.442	2.82	12.45	9.09	676
2.71	.402	2.56	10.73	8.01	583	3.36	.451	2.88	12.22	8.86	672
2.83	.439	2.80	11.08	8.26	600	3.36	.461	2.94	12.30	8.94	669
2.88	.494	3.15	10.91	8.03	604	3.36	.498	3.18	12.45	9.09	696
2.89	.460	2.93	11.08	8.19	602	3.37	.459	2.93	12.02	8.65	651
2.90	.405	2.58	11.25	8.35	605	3.37	.469	2.99	12.51	9.14	699
2.91	.443	2.83	11.12	8.21	609	3.38	.521	3.32	12.53	9.15	699
2.93	.458	2.92	11.80	8.88	629	3.39	.440	2.81	12.26	8.87	678
2.93	.397	2.53	11.56	8.63	625	3.40	.522	3.33	12.18	8.78	673
2.95	.395	2.52	11.35	8.40	613	3.40	.507	3.24	12.66	9.26	708
2.96	.412	2.63	11.57	8.62	619	3.40	.461	2.94	12.27	8.87	680
2.96	.421	2.69	11.52	8.56	618	3.42	.450	2.87	12.29	8.87	685
2.96	.425	2.71	11.39	8.43	622	3.43	.523	3.34	13.04	9.61	704
2.97	.418	2.67	11.31	8.34	611	3.43	.521	3.32	12.54	9.11	692
2.98	.475	3.03	11.84	8.86	644	3.43	.553	3.53	12.72	9.29	705
3.00	.434	2.77	11.84	8.84	645	3.44	.462	3.95	12.04	8.60	669
3.00	.473	3.02	11.31	8.31	617	3.48	.549	3.50	12.81	9.33	707
3.01	.443	2.83	11.72	8.72	631	3.51	.482	3.08	12.37	8.86	684
3.03	.398	2.54	11.44	8.41	620	3.53	.503	3.21	12.21	8.68	672
3.03	.427	2.72	11.48	8.45	643	3.54	.506	3.23	12.48	8.94	696
3.04	.439	2.80	11.68	8.63	631	3.59	.430	2.74	12.32	8.73	693
3.07	.462	2.95	11.88	8.82	646	3.60	.496	3.16	12.54	8.94	701
3.07	.421	2.69	11.65	8.58	640	3.61	.441	2.81	12.28	8.67	694
3.08	.433	2.76	11.65	8.57	632	3.64	.442	2.82	12.63	8.99	705
3.08	.487	3.11	11.72	8.65	630	3.66	.461	2.94	12.62	8.96	706
3.09	.440	2.81	12.19	9.10	662	3.66	.474	3.02	12.47	8.81	699
3.09	.441	2.81	11.76	8.67	643	3.70	.592	3.78	12.52	8.82	716
3.10	.492	3.14	12.13	9.03	658	3.71	.656	4.19	13.39	9.68	763
3.10	.439	2.80	11.85	8.75	642	3.73	.552	3.52	13.27	9.54	746
3.11	.488	3.11	11.46	8.35	630	3.73	.517	3.30	12.51	8.78	705
3.11	.426	2.72	11.82	8.70	640	3.74	.404	3.58	12.22	8.48	698
3.13	.398	2.54	11.71	8.58	633	3.76	.506	3.23	12.45	8.69	705
3.13	.435	2.78	11.69	8.56	647	3.76	.520	3.32	12.68	8.92	710
3.13	.466	2.97	12.07	8.94	657	3.76	.489	3.12	11.44	7.68	667
3.13	.432	2.76	11.54	8.41	638	3.76	.528	3.37	12.71	8.95	720
3.13	.466	2.97	12.11	8.98	661	3.78	.514	3.28	13.19	9.41	739
3.15	.446	2.85	11.97	8.82	657	3.79	.487	3.11	12.75	8.96	716
3.16	.466	2.97	12.42	9.26	670	3.79	.454	2.90	12.72	8.93	719
3.18	.441	2.81	12.19	9.10	658	3.79	.642	4.10	13.84	10.05	773
3.19	.414	2.64	11.60	8.41	637	3.81	.470	3.00	12.68	8.87	713
3.19	.502	3.20	11.75	8.56	646	3.84	.590	3.76	13.68	9.84	756
3.21	.477	3.04	11.90	8.69	651	3.84	.552	3.52	13.40	9.56	751
3.21	.481	3.07	11.78	8.57	645	3.86	.507	3.24	12.51	8.65	703
3.21	.503	3.21	12.00	8.79	664	3.87	.509	3.25	13.07	9.20	738
3.22	.492	3.14	12.25	9.03	672	3.87	.451	2.88	12.71	8.84	721
3.22	.487	3.11	11.74	8.52	644	3.92	.532	3.39	13.22	9.30	745
3.22	.391	2.50	11.73	8.51	646	3.95	.541	3.45	12.52	8.57	715
3.23	.451	2.88	12.25	9.02	667	3.95	.462	2.95	12.91	8.96	741
3.23	.465	2.97	11.81	8.58	661	3.98	.513	3.27	13.07	9.09	740
3.23	.474	3.02	12.04	8.81	658	4.00	.515	3.29	13.16	9.16	753
3.24	.450	2.87	11.85	8.61	651	4.01	.486	3.10	12.87	8.86	744
3.24	.428	2.73	11.82	8.58	649	4.04	.564	3.60	13.57	9.53	770
3.24	.448	2.86	11.93	8.69	652	4.11	.498	3.18	12.65	8.54	733
3.25	.429	2.74	11.81	8.56	651	4.13	.775	4.95	14.68	10.55	848
3.25	.480	3.06	11.64	8.38	658	4.16	.613	3.91	14.01	9.85	797
3.25	.496	3.16	11.92	8.67	664	4.18	.531	3.39	13.50	9.32	770
3.27	.491	3.13	11.82	8.55	653	4.20	.603	3.85	13.97	9.77	795
3.28	.466	2.97	11.93	8.65	645	4.27	.542	3.46	13.66	9.39	786
3.28	.513	3.27	12.63	9.35	673	4.29	.569	3.63	14.15	9.86	799
3.31	.467	2.98	12.03	8.72	667	4.41	.620	3.96	14.58	10.16	818
3.31	.485	3.09	11.86	8.55	666	4.46	.562	3.59	14.09	9.63	806
3.32	.533	3.40	12.48	9.16	691	4.52	.646	4.12	14.64	10.12	847
3.32	.456	2.91	11.91	8.59	668	4.60	.571	3.64	14.28	9.68	830

[a] Calorie here refers to a small calorie.

TABLE 2.—*Average composition of milk, from analyses given in Table 1, when the other components are arranged corresponding to intervals of 0.2 per cent of the mean fat content*

Fat range	Samples	Fat	Nitrogen	Protein	Solids	Solids not fat	Energy
Per cent	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Calories per gram*
2.60–2.80	6	2.70	0.424	2.71	10.85	8.15	586
2.80–2.99	13	2.93	.434	2.77	11.37	8.44	615
3.00–3.19	26	3.10	.447	2.85	11.79	8.69	643
3.20–3.39	34	3.29	.470	3.00	12.08	8.79	666
3.40–3.59	13	3.46	.498	3.25	12.46	9.00	690
3.60 3.79	18	3.72	.510	3.31	12.68	8.96	716
3.80–4.00	11	3.90	.513	3.27	13.00	9.10	734
4.01–4.19	6	4.11	.578	3.69	13.55	9.44	777
4.20–4.39	3	4.25	.571	3.65	13.93	9.68	793
4.40–4.60	4	4.50	.600	3.83	14.40	9.90	825

TABLE 3.—*Statistical constants derived from figures for analysis of 134 samples of milk shown in Table 1*

Item	Means	Standard deviations	Coefficients of correlation between fat and other components
Fat (f)	3.4093±0.0245	0.4210±0.0173	$r_{fs}=0.9553\pm0.00577$
Energy (e)	680.075±3.300	56.61±2.332	
Nitrogen (n)	.4590±.0035	.06014±.00248	$r_{fn}=.7027\pm.0295$
Solids (s)	12.269±.04821	.8264±.03405	$r_{fe}=.9089\pm.0100$
Solids not fat (t)	8.8600±.0278	.4771±.0197	$r_{ft}=.6919\pm.0304$
Solids not fat (t)			[a] $B_{fe}=-.476$
Energy (e)			[a] $B_{fs}=1.36$
Solids not fat and energy			[b] $R=.985$

[a] Partial correlation coefficients.
[b] Multiple correlation coefficient between fat, solids not fat, and energy.

RELATIONSHIP OF ENERGY AND FAT

The total energy of milk correlates more intimately with the fat content than does any of the other constituents; thus, Overman and Sanmann (*12*, *13*) found a positive correlation of 0.9813 ± 0.0017 between the fat percentage and energy value of 212 samples of milk from the dairy heard of the University of Illinois. The samples analyzed were 3-day composites of the milk of Ayrshire, Guernsey, Holstein-Friesian, Jersey, and crossbred Guernsey-Holstein Friesian cows at all stages of the lactation period. The milks ranged between 2.68 and 7.59 per cent fat. Direct energy determinations were made with a bomb calorimeter, and from the data collected Overman and Sanmann derived the equation $E=115.3277\ (2.5064+f)$ Calories per kilogram. (E is the energy expressed in large calories and f is the percentage of fat.)

Gaines (*5*) estimated energy values of milk on the basis of values of 4,220 calories per pound of fat, and 1,860 calories per pound of solids not fat, and reported correlations between energy and fat as high as 0.9912. From the data of Stocking and Brew (*18*) he proposed the equation $E=109.44\ (2.66+f)$ Calories per kilogram. From Hansson's data (*7*) he derived the equation $E=112.58\ (2.72+f)$, and from Andersen's data (*1*), the equation $E=113.49\ (2.64+f)$. Möllgaard (*10*) showed the energy value of milk to be a linear function of the fat content. He used 60 samples of milk from 46 Red Danish cows to derive the equation $E=115\ (2.44+f)$, and 11 samples from 11 Jersey cows to derive the formula $E=101\ (3.594+f)$.

From the data presented in this report the equation $E=128.55$ $(1.886+f)$ was derived; and by the employment of the values for solids not fat (t) was derived the regression equation $E=183$ $(f-0.31t+3.04)$.

A comparison of the determined energy values with those computed from several formulas is given in Table 4. The determined values are averages of the energy of those samples within ± 0.1 per cent of the given fat percentage. In view of the fact that the formulas used to calculate these data were derived from studies with different breeds, under diverse experimental conditions, and employing various methods of sampling, the computed energy values show remarkable agreement among themselves and with those that were directly determined. For milk containing more than 3.7 per cent of fat Gaines's formula gives increasingly low energy values with increasing fat content, Overman and Sanmann's formula gives values agreeing throughout within 2 per cent of the determined values. The agreement between the determined energy of milk and that computed from its fat percentage, together with the high coefficient of correlation of fat and energy values, shows that the energy value of milk can be estimated from its fat content with considerable accuracy.

TABLE 4.—*Comparison of determined energy values (E) of milk with those computed from various formulas at given fat percentages*

Derivation of E	Calories per kilogram of energy in milk containing the percentage of fat indicated									
	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.1	4.3	4.5
Kahlenberg and Voris, determined	586	615	643	666	690	716	734	777	793	824
Kahlenberg and Voris, formula, 128.55 (1.886+f)	590	615	641	667	692	718	744	770	795	821
Kahlenberg and Voris, formula, 183 (f−.31t+3.04)	587	607	630	664	686	725	754	770	794	814
Overman and Sanmann, formula, 115.3277 (2.5064+f)	600	624	647	670	693	716	739	762	785	808
Gaines, formula, 109.44 (2.66+f)	587	608	630	652	674	696	718	740	762	784
Andersen, formula, 113.49 (2.64+f)	606	629	651	674	697	720	742	765	788	810
Hansson, formula, 112.58 (2.72+f)	610	633	655	678	700	723	745	768	790	813
Möllgaard, formula for Red Danish cows, E=115 (2.44+f)	591	614	637	660	683	706	729	752	775	798
Möllgaard, formula, for Jersey cows, E=101 (3.594+f)	635	656	676	696	716	737	757	777	797	817

RELATION OF PROTEIN AND FAT

The secretion of protein in milk seems to be intimately related to that of fat. As far back as 1899 Timpe (*19*) derived the relation $P=2+0.35f$. The milk from which this formula was derived represented 21 cows of various breeds. In this formula and in those that follow P represents the percentage of protein and f the percentage of fat. Van Slyke (*20*) proposed the equation $P=1.6+0.4f$, from a large number of analyses of herd milk and of milk from individual cows of different breeds. Andersen and Langmack (*2*) in 1923 reported a study of 1,080 analyses of milk of Red Danish cows, from which the equation $P=1.597+0.446f$ was derived. Gaines (*4*), using Haecker's (*6*) published analyses of 543 samples of mixed milk, found a correlation coefficient existing between the fat and protein of $+0.812\pm0.010$. He proposed the equation $P=1.46+0.40f$. Overman and Sanmann (*12, 13*), working with 212 samples of mixed milk, reported a correlation coefficient of 0.792 ± 0.017. Each sample was a composite made from all of the milk produced by one cow during

three days, and taken at all stages of normal lactation periods. The equation calculated from their data is $P = 1.8816 + 0.3700f$. Later, these investigators (*14*) reported a coefficient of correlation of protein to fat of 0.727 ± 0.0194 for 268 samples of Holstein-Friesian milk. The samples were 3-day composites taken at 5-week intervals throughout the lactation periods of individual cows. Overman, Sanmann, and Wright (*14*) proposed the equation $P = 1.1004 + 0.6531f$. Other equations were reported by these authors for the milk of Ayrshire, Guernsey, Jersey and Guernsey-Holstein Friesian cows. The equation derived from the 1,998 milk samples of "all" breeds was given as $P = 1.9646 + 0.4059f$.

The equation derived from the data presented in this report is $P = 0.8977 + 0.6393f$, the correlation coefficient being 0.7027 ± 0.0295.

A comparison of the protein content of milk computed from this and from other equations is to be found in Table 5. It will be seen that the results given by Gaines's formula are appreciably lower than by any of the others. In milk of the lower fat percentages the values computed from Van Slyke's equation compare favorably with the determined and computed values here presented. In milk of fat content between 3.5 and 3.9 per cent the results from equations of Timpe, Andersen and Langmack, Overman and Sanmann, and those derived from the present study agree very closely. Differences between the breeds and conditions involved forbid close comparisons of formulas. The difference between the two Holstein-Friesian formulas (Overman, Sanmann, and Wright's and the one here presented) might possibly be explained by the fact that the formula of Overman and his collaborators (*14*) was derived from milk ranging in fat content from 2.6 to 6.24 per cent, while the formula here presented was derived from milk ranging in fat content from 2.66 to 4.60; also there were differences in the preparation of the composite samples of the milk.

TABLE 5.—*Comparison of determined values for protein (P) of milk with those computed from various equations at given fat percentages*

Derivation of P	Percentage of protein in milk containing the percentage of fat indicated									
	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.1	4.3	4.5
Kahlenberg and Voris, determined	2.71	2.77	2.85	3.00	3.18	3.25	3.27	3.69	3.65	3.89
Kahlenberg and Voris, Holstein-Friesian formula P=0.8977+0.6393f	2.62	2.75	2.88	3.01	3.14	3.26	3.39	3.52	3.65	3.78
Timpe, formula, P=2+0.35f	2.95	3.02	3.09	3.16	3.23	3.30	3.37	3.44	3.51	3.58
Van Slyke, formula, P=1.6+0.4f	2.68	2.76	2.84	2.92	3.00	3.08	3.16	3.24	3.32	3.40
Andersen and Langmack, formula, P=1.597+0.446f	2.80	2.89	2.98	3.07	3.16	3.25	3.34	3.43	3.52	3.60
Gaines, formula, P=1.46+0.40f	2.54	2.62	2.70	2.78	2.86	2.94	3.02	3.10	3.18	3.26
Overman and Sanmann, formula, P=1.8816+0.3700f	2.88	2.96	3.03	3.10	3.18	3.25	3.33	3.40	3.47	3.55
Overman, Sanmann, and Wright, Holstein-Friesian formula, P=1.1004+0.6531f	2.86	2.99	3.13	3.26	3.39	3.52	3.65	3.78	3.91	4.04
Overman, Sanmann, and Wright, "all-breeds" formula, P=1.9646+0.4059f	3.06	3.14	3.22	3.30	3.39	3.47	3.55	3.63	3.71	3.79

RELATION OF TOTAL SOLIDS AND FAT

Frequent use has been made of equations for computing the percentage of total solids of milk, from the specific gravity and the percentage of fat in the milk (*11*), but not so many studies have been

made of the relation of the fat percentage and the total solids. Lythgoe (*9*) pointed out a direct relationship between the fat percentage and solids. Overman and Sanmann (*12, 13*) obtained a coefficient of correlation of 0.9640 ± 0.0033 between the total solids and the fat percentage. The equation calculated from their data is $TS = 1.414\ (5.13 + f)$. Later, these investigators (*14*) obtained similar equations for the milk of the different breeds. For Holstein-Friesians, they reported a coefficient of correlation of 0.8750 ± 0.0097, and for "all" breeds a correlation of 0.9220 ± 0.0023. The derived equation for the milk of the Holstein-Friesian breed was $TS = 1.594\ (4.29 + f)$, and for the total milk samples of "all" breeds, $TS = 1.395\ (5.44 + f)$. Möllgaard (*10*) derived the relationship $TS = 1.346\ (5.67 + f)$.

The equation derived from the data presented in this report is $TS = 1.783\ (3.47 + f)$, with a coefficient of correlation equal to 0.9089 ± 0.0100.

TABLE 6.—*Comparison of determined values for total solids (TS) of milk with those computed from various formulas at given fat percentages*

Derivation of TS	Percentage of total solids in milk containing the percentage of fat indicated									
	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.1	4.3	4.5
Kahlenberg and Voris, formula, determined	10.85	11.37	11.79	12.08	12.46	12.68	13.00	13.55	13.93	14.44
Kahlenberg and Voris, formula, TS = 1.783 (3.47+f)	11.00	11.36	11.71	12.07	12.43	12.78	13.14	13.50	13.85	14.21
Overman and Sanmann, formula, TS = 1.414 (5.13+f)	11.07	11.35	11.64	11.92	12.20	12.49	12.77	13.05	13.33	13.62
Overman, Sanmann, and Wright, Holstein formula, TS = 1.594 (4.29+f)	11.14	11.46	11.78	12.10	12.42	12.74	13.05	13.37	13.69	14.01
Overman, Sanmann, and Wright, "all-breeds" formula, TS = 1.395 (5.44+f)	11.36	11.63	11.91	12.19	12.47	12.75	13.03	13.31	13.59	13.87
Möllgaard, formula, TS = 1.346 (5.67+f)	11.27	11.54	11.80	12.07	12.34	12.61	12.88	13.15	13.42	13.69

A comparison of the total solids computed from the various equations at given fat percentages is given in Table 6. The agreement between the results of all formulas is within 5 per cent. The values computed by means of Overman, Sanmann, and Wright's formula, specific for the Holstein-Friesian breed, practically agree throughout with the computed and determined values here presented. The total solids, therefore, may be computed with reasonable accuracy from the percentage of fat in the milk.

SUMMARY

In a statistical study of the relationships between the fat percentage and the energy, the protein, the solids not fat, and the total solids in 134 samples of Holstein-Friesian milk representing one entire lactation period of 12 cows, the following correlation coefficients were obtained; fat with energy 0.9553 ± 0.0058; fat with protein 0.7027 ± 0.0295; fat with total solids, 0.9089 ± 0.0100; fat with solids not fat 0.6919 ± 0.0304. The multiple correlation coefficient for energy with fat and solids not fat is 0.985.

The regression equation of energy on fat is $E = 128.55\ (1.886 + f)$; of protein on fat, $P = 0.8977 + 0.6393f$; of total solids on fat, $TS = 1.783\ (3.47 + f)$; for solids not fat on fat, $t = 0.7841\ (7.8903 + f)$, for energy on fat and solids not fat, $E = 183\ (3.04 - .31t + f)$.

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